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# Metabolic and Behavior Changes in Surubim Acutely Exposed to a Glyphosate-Based Herbicide

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**Abstract** This study examined the effect of glyphosate-based herbicide (Roundup Original), the major herbicide used in soybean crops in Mato Grosso state, at concentrations of 0, 2.25, 4.5, 7.5, and 15 mg $L^{-1}$ on metabolic and behavior parameters of the hybrid fish surubim in an acute exposure lasting 96 h. Glycogen content, glucose, lactate, and protein levels were measured in different tissues. Plasma levels of cholesterol, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were also determined. Ventilatory frequency (VF) and swimming activity (SA) were considered behavior parameters. Results showed that herbicide exposure decreased plasma glucose levels and increased it in surubim liver. Lactate increased in both plasma and liver but decreased in muscle. Protein levels decreased in plasma and muscle but increased in liver. After herbicide exposure, liver and muscle glycogen was decreased. Cholesterol levels decreased in plasma at all concentrations tested. Plasma ALT increased, and no alterations were recorded for AST levels. VF increased after glyphosate exposure (5 min) and decreased after 96 h. SA showed differences among all groups (5 min). At the end of 96 h, SA was altered by the 7.5 mg $L^{-1}$ concentration. Fish used anaerobic glycolysis as indicated by generally decreased glycogen levels and decreased lactate levels in muscle but increased ones in plasma and liver. We suggest that the studied parameters could be used as indicators of herbicide toxicity in surubim and may provide extremely important information for understanding the biology of the animal and its responsiveness to external stimuli (stressors).

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Several hundred kinds of pesticides with different chemical structures are used worldwide in agriculture. Although these pesticides are considered to be essential for agricultural development, some can cause serious environmental contamination, mainly in water (Zanella et al. 2002). These agents are used against pests, undesirable herbs, and agricultural diseases, but they have adverse effects on the aquatic environment (Sarikaya & Yilmaz 2003). Biochemical parameters of blood and various tissues are used to identify biological alterations caused by pesticides in fish, including evaluation of enzymes, physiological alterations, and changes in behavior (Jyothi and Narayan 1999; Miron et al. 2005). According to Barton (2002) and Wendelaar-Bonga (1997), stress responses can involve both motor and neurovegetative reactions, which are mediated by the neuroendocrine and sympathetic nervous

systems, resulting in alertness response and increased energy mobilization, a condition in which homeostasis is disturbed by many internal and external factors named "stressors." Sarikaya and Yilmaz (2003) reported behavioral alterations in *Cyprinus carpio* L. when they were exposed to 2,4-dichlorophenoxyacetic acid. In addition, Langiano and Martinez (2008) observed that *Prochilodus lineatus*, when exposed to Roundup at sublethal concentrations, presented several histological, biochemical, and physiological modifications.

Glyphosate [*N*-(phosphonomethyl)glycine] is a nonselective, postemergence herbicide extensively used in various applications for weed and vegetation control. Numerous commercial formulations containing glyphosate as the active ingredient are becoming increasingly popular all over the world not only due to its high herbicidal activity but also because of its low mammalian toxicity (Corbera et al. 2005). The Roundup formulation contains glyphosate as the active ingredient with polyethoxylene amine, a nonionic surfactant, added to increase the efficiency of the active ingredients by promoting penetration of the herbicide through the plant cuticle (Brausch and Smith 2007; Lushchak et al. 2009). The toxicity and risk for humans, other mammals, and birds were analyzed in detail by Williams et al. (2000) who concluded that "under present and expected conditions of use, Roundup herbicide does not present a health risk for humans." However, some investigators have shown that aquatic organisms, particularly fish, could be more sensitive to glyphosate compared with mammals (Lushchak et al. 2009). Roundup formulation affected energy metabolism, hematological parameters, histological morphology, free-radical processes, and acetylcholinesterase activity in several fish species (Langiano & Martinez 2008; Lushchak et al. 2009; Glusczak et al. 2006, 2007, 2011; Menezes et al. 2011).

In the Mato Grosso state, the main herbicides used are based on glyphosate to control pests in crops that are genetically modified (INDEA-MT 2008–2009). This state is the major soybean transgenic producer in Brazil, and the use of pesticides in monocultures could be an important factor contributing to environmental pollution (Alegria & Shaw 1999). The north of Mato Grosso is part of the so-called Legal Amazon, a region of transition between forest and Cerrado, bathed by many rivers. Catch of native fish species is the reason for decreased populations of such. Fish farms have invested in the hybridization of native species, thus contributing to sustainable economic activity in the region. The hybrid fish, popularly called "surubim," is a result of artificial cross-breeding between two large Neotropical catfish species, pintado (*Pseudoplatystoma corruscans*) and cachara (*P. reticulatum*). This hybrid is considered an important source of dietary protein because of its favorable organoleptic characteristics, such as a better carcass, yield and meat with a particularly pleasant mild taste. The surubim was chosen for this study because the knowledge of herbicide effects on hybrid fish species in Mato Grosso is scarce. Although the potential environmental and human contamination by pesticides used in the production of grain and cotton in the state of Mato Grosso, as well as their impacts on the biota, is presumed, studies to characterize this impact are missing. Considering that there is no information available about Roundup Original toxicity and its adverse effects on this fish of commercial importance in the state of Mato Grosso (Brazil). The aim of the present study was to investigate the effect of Roundup Original through evaluation of several metabolic and behavioral parameters in hybrid surubim. The results of this study may provide information concerning the use of indicators to evaluate the sublethal toxicity of Roundup Original on hybrid fish.

## Materials and Methods

### Chemicals

The herbicidal product used in this study was glyphosate-based (Roundup Original, 480 g $L^{-1}$ containing isopropylamine salt of glyphosate (360 g $L^{-1}$) acid equivalent, N-(phosphonomethyl) glycine (glyphosate), and 684 g $L^{-1}$ of inert ingredients. Bovine serum albumin and paraphenylphenol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Other reagents used in the experiments were of the highest analytical grade (Aldrich, Merck, 98 to 99 %) by Germany, India, Japan and France. The stock solution of herbicide (100 mg $L^{-1}$) was prepared in water.

### Animals

Juvenile surubim (55.5 ± 10.0 g weight and 17.0 ± 2.0 cm length) were obtained from the fish farm Nativ (Sorriso, MT, Brazil). Before the beginning of the experiments, surubim were acclimated during 10 days to laboratory conditions in 300-L tanks with dechlorinated and aerated tap water. During this time they were fed once a day with commercial fish food containing 42 % crude protein (Supra, Brazil). Feces and pellet residues were removed every other day by suction.

### Toxicity Tests

After the acclimation period, the fish were distributed among the nonexposure (control) or four exposure groups (2.25, 4.5, 7.5, and 15 mg $L^{-1}$ of Roundup Original) for 96 h according to Glusczak et al. (2006). These concentrations were chosen

based on several other studies that also investigated the effects of the acute exposure of other fish species to herbicide using similar concentrations (Glusczak et al. 2006; Langiano and Martinez 2008). The fish were placed in 50-L glass tanks filled with dechlorinated tap water. The aquaria were continuously aerated and contained five fish per tank. The ratio of biomass (w/v) was chosen according to Aguiar et al. (2004) and Moraes et al. (2011). The assays were performed in triplicate ($n = 15$). Fish did not receive food during Roundup Original acute exposure (Glusczak et al. 2006; Lushchak et al. 2009; Modesto and Martinez, 2010). The herbicide was carefully added to the water from the stock solution (100 mg $L^{-1}$) only at the beginning of the experiment using hard and efficient aeration to distribute evenly in the tank. Throughout the experimental period, water-quality parameters (i.e., temperature, pH, dissolved oxygen, hardness, nonionized ammonia and nitrite) were monitored. After glyphosate exposure, surubim were removed from the aquarium, immediately anesthetized with benzocaine (0.08 g $L^{-1}$), and caudal vein blood drawn with a heparinized syringe. Fish were killed by punching the spinal cord behind the opercula then measured and weighed, and liver and muscle were quickly removed, washed with 150 mM NaCl, and frozen at −85 °C for further analysis. This study was approved by the Committee guidelines (Ethics in Animal Research of the Federal University of Mato Grosso), reference number 23108.053066/10-7.

## Chemical Analysis

Concentrations of glyphosate were measured at the beginning and the end of experimental period. The analyses were made by a coupled-column liquid chromatography system with fluorescence detection. This method was applied after water derivatization with fluorescent reagent 9-fluorenylmethylcloroformate (FMOC). A first short C18 column (3 cm) was used to perform large-volume injection (2 mL) and effect the efficient separation between the derivatized analytes and the excess of FMOC. It was coupled to a second amino analytical column (25 cm) for anion-exchange separation of the derivates. The limit of quantification was 0.1 μg $L^{-1}$ (without preconcentration) or 0.02 μg $L^{-1}$ (after preconcentration with 50 mL of water sample using anionic resin) (Hidalgo et al. 2004).

## Determination of Metabolic Parameters

Liver and muscle glycogen contents were determined according to the method of Bidinotto et al. (1997). The samples were solubilized with 6.0 N KOH in a boiling water bath (approximately 100 °C) for 5 min, and the glycogen was precipitated by ethanol and $K_2SO_4$ saturated solution. After centrifugation at 2,000 *g* for 3 min, the supernatants were discarded and the pellets resuspended in distilled water; he glycogen content was determined as glucosyl-glucose by phenol–sulfuric acid (Duboie et al. 1956). For protein quantification, the tissues were heated with 6.0 N KOH at 100 °C and centrifuged at 1,000 *g* for 10 min. The supernatant was used to determine total protein content according to the method described by Lowry et al. (1951). Glucose and lactate from liver and muscle were photometrically quantified; the tissues were homogenized in a T10 Basic-IKA homogenizer with 10 % trichloroacetic acid and centrifuged at 1,000 *g* for 10 min for flocculation of the proteins. The deproteinated supernatant was used for the determinations of lactate at 570 nm (Harrower and Brown 1972), and glucose was determined as decreasing sugar at 480 nm (Duboie et al. 1956).

Blood samples were centrifuged (for 5 min at 5,000 *g*), and the blood plasma was stored at −85 °C until further analysis. Plasma glucose and total protein levels were analyzed using colorimetric commercial kits based on the glucose oxidase method (Labtest, Lagoa Santa, Minas Gerais, Brazil) and biuret (Labtest) reactions, respectively. Plasma lactate and cholesterol levels were analyzed using enzymatic commercial kits (Labtest) based on lactate oxidase–peroxidase and esterase-oxidase reactions, respectively. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using commercial kits (Labtest) based on kinetic Ultraviolet-International Federation of Clinical Chemistry and Laboratory Medicine (UV-IFCC) reactions.

## Behavioral Parameters

### *Acclimation Period*

Surubim were acclimated to laboratory conditions for 10 days in 50-L tanks with dechlorinated and aerated tap water. During this time they were fed once a day with commercial fish food containing 42 % crude protein (Supra, Brazil). Feces and pellet residues were removed every day by suction to avoid changes in water quality.

After acclimation, 30 fish were distributed into 5 groups [0 (control), 2.25, 4.5, 7.5, and 15 mg $L^{-1}$ of Roundup Original (Monsanto, São José dos Campos/SP, Brazil)] containing 2 fish/aquarium. Tests were performed in triplicate, and surubim did not receive food during 96-h exposure (the experimental period). The herbicide was carefully added to the water from the stock solution (100 mg $L^{-1}$) only at the beginning of the experiment using hard and efficient aeration to distribute evenly in the tank.

## Ventilatory Frequency

Observations by video were recorded through a small opening in a black curtain, which was placed in front of all aquaria to avoid disturbing the fish and prevent the observer

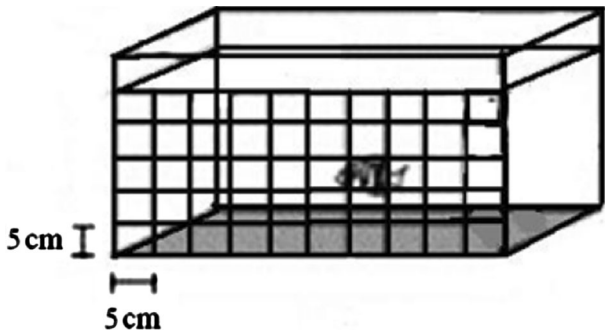


**Fig. 1** Plotted aquarium in *squares* (5 cm/side) to measure SA

from being seen. The walls of the glass aquarium were externally covered by cardboard partitions on three sides (except for the front) to improve observation and recording. Five minutes after addition of the herbicide to the aquarium, fish behavior was recorded for 5 min; at the end of the experiment (after 96 h), behavior recorded for another 5 min. The technique used to quantify ventilatory frequency (VF) was the counting of each opercular or buccal movement (beats per minute) seen in the video record.

*Swimming Activity or Locomotion*

Observations by video were recorded through a small opening in a black curtain to avoid disturbing fish. The walls of the aquarium were externally covered by partitions on three sides except on the front wall, on which was plotted squares of 5 cm/side (Fig. 1). Fish positions were recorded, and displacement was calculated by determining the fish's eye position, which means that when a fish did display any movement, the focus was on its eyes, i.e., when the fish moved, the observer measured how many squares it swam or moved inside the aquarium. When a fish displayed swimming activity (SA), its eyes were the point by which displacement was calculated in centimeters (Fig. 1). The activity of fish, either normal or hyperactive SA, was observed by recording the fish in each tank for 5 min at two time periods: (1) at 5 min after Roundup Original addition and (2) at 96 h after herbicide exposure. After the recordings were made, the observer analyzed the videos to count the displacement of each fish during two the periods of the experiment. Each fish was evaluated individually ($n = 6$ fish/group), and displacement means were measured in centimeters.

Statistical Procedures

For metabolic parameters, the mean ± SD of the mean (SDM) of all groups was calculated. After the determination of normal distribution (Kolmogorov–Smirnov test) and homogeneity of data variance (Bartlett's test), analysis of variance (ANOVA) was performed. Differences between each treatment against the control group were detected by applying Dunnett's test. Behavioral data were analyzed by nonparametric (Kruskal–Wallis-test) ANOVA followed by Dunn's or Wilcoxon sign test (for post hoc between groups and within-groups comparisons, respectively) (GraphPad Prism 5.00). Data are presented as median and interquartile range, and $p < 0.05$ was considered significant. Water parameters were determined by mean ± SDM.

**Table 1** Measurement of aqueous concentrations of glyphosate (mg $L^{-1}$) at the beginning (0 h) and end (96 h) of the herbicide exposure (mean ± SD); quantified by HPLC following of % of glyphosate (Gly) reduction

| Time of exposure (h) | Glyphosate (2.25) | % Reduction (Gly) |
|---|---|---|
| 0 | 2.24 ± 0.05 | 0 |
| 96 | 2.01 ± 0.03 | 10.26 |
| Time of exposure (h) | Glyphosate (4.5) | |
| 0 | 4.52 ± 0.06 | 0 |
| 96 | 3.85 ± 0.04 | 14.82 |
| Time of exposure (h) | Glyphosate (7.5) | |
| 0 | 7.55 ± 0.07 | 0 |
| 96 | 6.64 ± 0.05 | 12.05 |
| Time of exposure (h) | Glyphosate (15) | |
| 0 | 14.8 ± 0.09 | 0 |
| 96 | 13.9 ± 0.085 | 6.08 |

## Results

Chemical Measurements and Abiotic Parameters

Water conditions during exposure were as follows: temperature 26 ± 1.0 °C, pH 6.75 ± 0.4, dissolved oxygen 6.31 ± 0.5 mg $L^{-1}$, hardness 18 ± 2.0 mg $L^{-1}$ $CaCO_3$, nonionized ammonia 0.9 ± 0.01 μg $L^{-1}$, and nitrite 0.06 ± 0.01 mg $L^{-1}$. Water quality did not change during the experimental period. Aqueous concentrations of glyphosate were determined at the beginning (time 0 h) and at the end (time 96 h) of exposure (Table 1). The water glyphosate concentration ranged from 2.01 up to 13.9 mg $L^{-1}$, and the glyphosate decreases were 10.26, 14.82, 12.05, and 6.08 % to 2.25, 4.5, 7.5, and 15 mg $L^{-1}$, respectively.

As listed in Table 2, apparent signs of poisoning were observed in surubim after glyphosate-based herbicide exposure at the tested concentrations. The 15 mg $L^{-1}$ concentration caused mortality in 50 % of fishes during 96 h of glyphosate exposure, although the other concentrations did not cause mortality.

Metabolic Parameters

Surubim exposed to glyphosate exhibited significant decreases in both liver (2.25 and 4.5 mg $L^{-1}$

**Table 2** Levels of the biochemical metabolites in plasma, liver, and muscle of surubim exposed to Roundup Original for 96 h

| | | Control | 2.25 mg $L^{-1}$ | 4.5 mg $L^{-1}$ | 7.5 mg $L^{-1}$ | 15 mg $L^{-1}$ |
|---|---|---|---|---|---|---|
| Glycogen (μmol $g^{-1}$ tissue) | Liver | 42.14 ± 9.72 | 24.04 ± 5.96[a] | 24.73 ± 5.53[a] | 41.79 ± 9.70 | 31.43 ± 6.90 |
| | Muscle | 7.61 ± 0.52 | 5.77 ± 0.62[a] | 7.32 ± 1.05 | 8.40 ± 0.64 | 8.09 ± 1.53 |
| Glucose (mmol $g^{-1}$ tissue/mg $dL^{-1}$ plasma) | Plasma | 43.47 ± 3.68 | 39.00 ± 6.60 | 34.75 ± 3.33[a] | 39.42 ± 4.09 | 36.11 ± 5.90[a] |
| | Liver | 0.05 ± 0.01 | 0.05 ± 0.01 | 0.22 ± 0.03[a] | 0.15 ± 0.02[a] | 0.20 ± 0.03[a] |
| | Muscle | 0.02 ± 0.004 | 0.02 ± 0.003 | 0.02 ± 0.003 | 0.02 ± 0.002 | 0.02 ± 0.004 |
| Lactate (μmol $g^{-1}$ tissue/mg $dL^{-1}$ plasma) | Plasma | 14.97 ± 1.51 | 19.45 ± 2.52 | 13.03 ± 3.53 | 22.22 ± 5.00[a] | 22.12 ± 4.34 |
| | Liver | 6.04 ± 1.45 | 6.16 ± 0.59 | 6.76 ± 1.31 | 8.43 ± 0.90[a] | 9.53 ± 1.60[a] |
| | Muscle | 4.39 ± 0.61 | 4.12 ± 0.78 | 3.91 ± 0.66 | 3.18 ± 0.28[a] | 3.83 ± 0.77 |
| Protein (mg $g^{-1}$ tissue/mg $mL^{-1}$ plasma) | Plasma | 30.31 ± 6.76 | 24.25 ± 4.13 | 18.00 ± 3.82[a] | 25.43 ± 4.53 | 24.34 ± 3.55 |
| | Liver | 228.10 ± 46.92 | 246.00 ± 26.88 | 244.30 ± 42.94 | 377.20 ± 74.53[a] | 477.10 ± 91.17[a] |
| | Muscle | 88.70 ± 6.25 | 90.76 ± 12.22 | 52.47 ± 5.50[a] | 54.07 ± 7.95[a] | 98.84 ± 4.50 |
| Cholesterol (mg $dL^{-1}$ plasma) | Plasma | 92.90 ± 17.47 | 69.25 ± 16.54[a] | 62.79 ± 12.15[a] | 65.28 ± 12.37[a] | 58.14 ± 10.64[a] |

Data represent mean ± SDM ($n = 7$)

[a] Difference between groups and control values (ANOVA followed by Dunnett test), $p < 0.05$

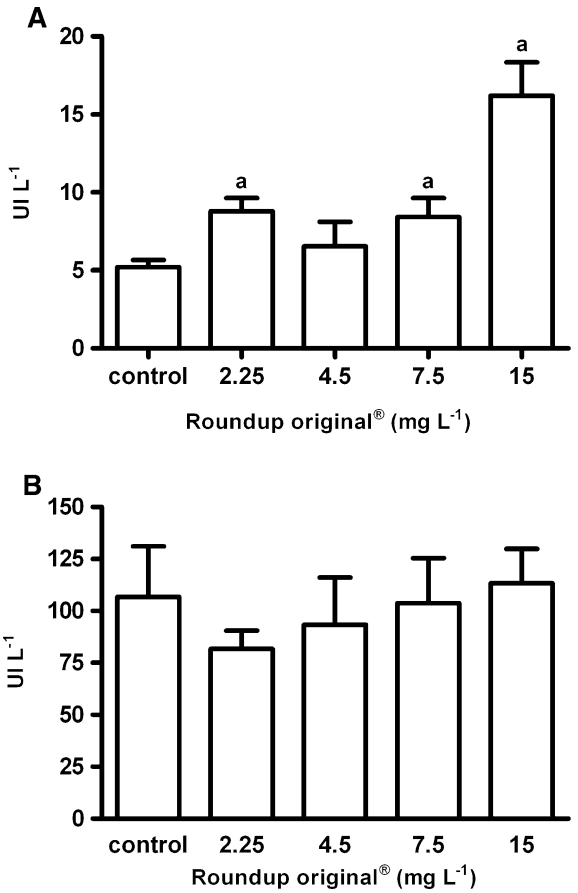


**Fig. 2** Transaminases activities in plasma of surubim exposed to 2.25, 4.5, 7.5, and 15 mg $L^{-1}$ Roundup Original or only water (control) for 96 h. **a** ALT activity. **b** AS activity. Data are means ± SDM ($n = 7$). [a]Difference from respective control, $p < 0.0001$ (ANOVA followed by Dunnett's test)

concentration) and muscle glycogen content (2.25 mg $L^{-1}$ concentration) compared with control fish (Table 2). Glucose levels significantly decreased in plasma (4.5 and 15 mg $L^{-1}$ concentrations), but in liver there was a significant increase at concentrations of 4.5, 7.5, and 15 mg $L^{-1}$. Muscles exhibited different responses to metabolic parameters and no variations were recorded to glucose (Table 2).

Levels of lactate significantly increased in plasma (7.5 and 15 mg $L^{-1}$ concentrations) and in liver (7.5 and 15 mg $L^{-1}$ concentrations) but were significantly decreased in muscle (7.5 mg $L^{-1}$ concentration) compared with control fish (Table 2). Protein levels significantly decreased in both plasma (4.5 mg $L^{-1}$ concentration) and muscle (4.5 and 7.5 mg $L^{-1}$ concentrations). Liver showed a significant increase in protein levels (7.5 and 15 mg $L^{-1}$ concentrations) during the exposure period (Table 2). Plasma cholesterol levels significantly decreased at all tested concentrations (Table 2). Surubim exposure to Roundup Original promoted a significant increase in plasma ALT activity at the 2.25, 7.5, and 15 mg $L^{-1}$ concentrations (Fig. 2a), but plasma AST activity was not altered (Fig. 2b).

## Behavioral Analysis

### *Ventilatory Frequency (VF)*

For behavior analyses, the results showed that 5 min after herbicide exposure, VF values were significantly greater for fish exposed to 7.5 and 15 mg $L^{-1}$ herbicide (205 ± 26 and 203 ± 28 bpm, respectively) than for fish from the control group (86 ± 4 bpm). After 96-hour Roundup

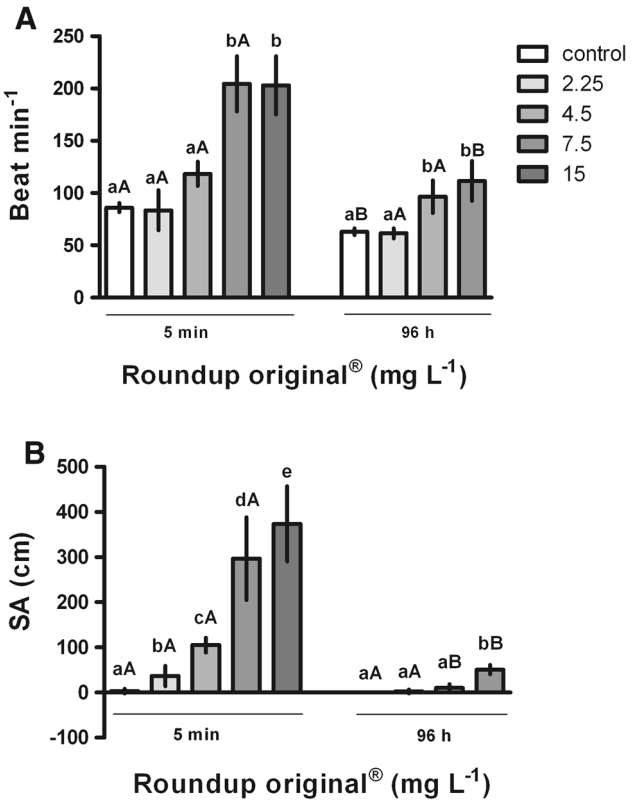


**Fig. 3** Behavioral parameters of surubim exposed to herbicide for 5 min and 96 h in different concentrations of Roundup Original. **a** VF. **b** SA. Data are reported as median and interquartile range ($n = 6$). *Different lower-case letter* indicate statistic difference between groups in the exposure period (5 min or 96 h) (Kruskal–Wallis-test followed by Dunn's test, $p < 0.05$. Capital letters indicate statistical difference for each concentration in both exposure periods (5 min and 96 h) (Wilcoxon sign test for paired data, $p < 0.05$). Fish exposed to 15 mg $L^{-1}$ herbicide died 12 h after exposure ($n = 6$)

Original exposure, the 4.5 and 7.5 mg $L^{-1}$ concentrations led to increasing values of VF (97 ± 16; 112 ± 19 bpm, respectively) compared with the control group (63 ± 3 bpm). When comparing VF during two distinct periods, i.e., at the beginning (5 min) and the end (96 h) of the experiment in the same group, VF control values at 5 min were significantly greater (86 ± 4 bpm) than those after 96 h (63 ± 3 bpm), although these values are within the range of normal for surubim VF. Fish exposed to 7.5 mg $L^{-1}$ of glyphosate also showed the same profile, i.e., increased VF values during 5 min (205 ± 26 bpm) compared with values from 96 h (112 ± 19 bpm) (Fig. 3a).

*Swimming Activity or Locomotion (SA)*

SA or locomotion in surubim exposed to herbicide showed differences among all groups in the first 5-minute analysis, and only the 7.5 mg $L^{-1}$ concentration promoted significant alterations in SA (0 ± 0; 51 ± 10 cm) after 96 h of herbicide exposure. When comparing SA during two periods, at the beginning (5 min) and at the end (96 h) of the experiment, surubim exposed to 4.5 and 7.5 mg $L^{-1}$ of herbicide showed a significant increase in SA values at 5 min (105 ± 16 and 297 ± 91 bpm) compared with those at 96 h (10 ± 9 and 51 ± 10 bpm, respectively; Fig. 3b).

In this study, only four groups were used [0 (control), 2.25, 4.5, and 7.5 mg $L^{-1}$ of glyphosate-based herbicide] because all fish exposed to the 15 mg $L^{-1}$ concentration died 12 h after the beginning of experiment (Fig. 3a, b), but no other concentration caused mortality during behavior evaluation.

## Discussion

The present study aimed to investigate the major herbicide, Roundup Original, used in soybean transgenic crops in northern Mato Grosso and its effects on hybrid fish surubim. This fish has undergone a hybridization process and is financially profitable to fish farmers due to its good acceptance in the food market. It is produced in fish farms installed around soybean monocultures that use glyphosate-based herbicide. Mato Grosso state is the largest soybean producer in Brazil and is emerging in aquaculture. For this reason, this study evaluated environmental pollution using the surubim as a toxicological bioindicator. Interestingly, the residual glyphosate measured in water showed a decrease of approximately 11 % compared with initial measured concentrations. Considering that this percentage of decrease was observed by comparing it twice (exposure period), the loss of herbicide suggests a possible absorption by fish tissues. However, more studies are necessary to investigate glyphosate and its metabolites in water and fish tissue to confirm this hypothesis. In general, the bioaccumulation of pesticides—such as the presence of glyphosate, which was detected in *Cyprinus carpio* (Abrantes et al. 2010) and in the fillets and fish eggs exposed to Roundup (Rendon-von Osten et al. 2005)—in tissues of aquatic animals can render them harmful for human consumption (Elia et al. 2006),

In our study, we used several glyphosate-based pesticide concentrations (2.25–15 mg $L^{-1}$) that were previously used in the literature showing that it induces increased energy metabolism, abnormal hematological parameters, and alterations in histological morphology as well as causes oxidative stress in several fish species (Glusczak et al. 2006; Langiano and Martinez 2008; Lushchak et al. 2009; Modesto and Martinez 2010; Cattaneo et al. 2011). These concentrations might be considered environmentally realistic considering that at current application rates, a water body with no intercepting vegetation can have a maximum concentration of 3.7 mg glyphosate $L^{-1}$, which

corresponds to 9 mg of Roundup $L^{-1}$ (Giesy et al. 2000); however, in the state of Mato Grosso the maximum concentration can be greater than that other states due to indiscriminate pesticide application. Our results showed that the greatest concentration (15 mg $L^{-1}$) caused mortality in 50 % of fish exposed to the herbicide. The $LC_{50}$ values (mg $L^{-1}$) for rainbow trout were between 8.2 and 27 for Roundup (Giesy et al. 2000); *Leporinus obtusidens* survived even at the greatest concentrations of Roundup tested [100 mg $L^{-1}$ (48 % acid equivalent)] (Glusczak et al. 2006). These results are consistent with those of Jiraungkoorskul et al. (2002), who observed a variation in the $LC_{50}$ = 2–55 mg $L^{-1}$ in different fish species, life stages, and test conditions after Roundup exposure. Our results could be a clear indication that the hybrid fish serubim is less resilient to glyphosate-based herbicide exposure than other fish species studied (after 96 h of exposure).

Behavioural changes are considered to be sensitive indicators of pesticide exposure (Miron et al. 2005). In the present study, fish increased their VF and SA in the first 5 min of herbicide exposure and then decreased them after 96 h. The decrease in VF and SA values after 96 h of exposure was likely an adapted response to stress (Menezes et al. 2011; Suarez and Mommesen 1987). VF is inversely proportional to fish body size (Schmidt-Nielsen 1996). The increased SA observed in surubim in the first 5 min of herbicide exposure can be considered hyperactivity compared with the SA of the control group. It means that surubim behaviour activities changed with increased VF and SA (or locomotion). In surubim, the resting frequency is approximately 63–87 bpm with almost no locomotion or SA during the day because it displays nocturnal activity. This clearly indicates that VF and SA are sensitive to disturbances, albeit of limited use, because these parameters do not reflect the severity of the stimulus (Barreto and Volpato 2004). Other studies, using different stressors, reported an increased average VF in Nile tilapia in response to confinement (Barreto and Volpato 2004) and to social stressors (Volpato et al. 1989). Overall, our results suggest that Roundup Original can adversely affect fish behaviour.

In the present study, Roundup Original led to a decreased liver glycogen level in surubim. These results are similar to previous studies by Moraes et al. (2011), Pretto et al. (2011), Shiogiri et al. (2012) showing that Roundup Original contributes as an energy source to increase behavioral activity such as VF and SA. Surubim also presented decreased levels of lactate and protein in muscle tissue: Probably because of stress conditions, the fish realized anaerobic glycolysis and muscle protein catabolism, thus consequently increasing VF and SA as a response. In the same way the herbicide promoted alteration in biochemistry parameters in plasma, such as decreased glucose, protein, and cholesterol levels and increased lactate levels. This shows that Roundup Original induces a metabolic alteration in fish by decreasing their energy sources, steroid hormone precursors, and cell membrane lipid component. One reasonable explanation for the decrease in the glycemia levels can be attributed to rapid tissue glucose consumption during hyperexcitability, such as VF and SA changes, which were evaluated as altered behaviour parameters caused by chemical toxicity in fish.

The liver is the most important organ to synthesize and store glycogen in the organism for blood glucose levels maintenance as well as a detoxifying tissue of xenobiotics (Suarez and Mommesen 1987). Glycogen content was also decreased in liver despite the increase in glucose, lactate, and protein levels. This probably occurred because the hepatic tissue performed glycogenolysis accompanied by activation of gluconeogenesis from lactate originating from intense muscular activity under hypoxic conditions (Fonseca et al. 2008). Because there was an increase of ALT activity in plasma, we think that hepatocytes may have suffered some injury/rupture due to the use of Roundup Original, which resulted in hepatic damage and ALT increase in the blood. In contrast, AST plasma activity did not change. The maintenance of normal plasma AST levels after 96 h of herbicide exposure may be a consequence of the short time exposure, which may have been insufficient to promote changes in hepatocytes, thus restricting the release of these enzymes in blood circulation. Considering that AST is an enzyme usually located in the liver mitochondria, it can be suggested that longer periods of herbicide exposure would be necessary to promote a damage that affects the mitochondrial membranes in a level high enough to release AST into the blood (Aguiar et al. 2004). Similarly, no alteration was observed in plasma AST of matrinxã (*Brycon cephalus*) after 96 h of Folidol 600 exposure and in *Rhamdia quelen* exposed to glyphosate (Aguiar et al. 2004; Ferreira et al. 2010). We can conclude that increased protein liver levels in fish after herbicide exposure could be related to repairs in hepatic cells due to toxic-agent action. It seems to represent an adaptive response against a possible tissue protein loss after herbicide exposure, which could increase hepatic protein synthesis. Other studies have shown that toxic stress causes an increase in protein levels because the synthesis of new proteins occurs to repair damaged cells or to replace lost enzymes due to tissue damage (Pretto et al. 2011; Oruç and Uner 1998). The impairment of both biochemical and physiological functions, along with behavioural changes of surubim, may be taken as indicators of negative effects caused by Roundup Original. In our study, it was observed that the increase of VF and SA is in accordance with the decrease of protein and glycogen contents in muscle in the attempt to maintain homeostasis.

## Conclusion

Biochemical and behavioural parameters evaluated in the present study could be used for monitoring the short-term effects of Roundup Original in the hybrid fish surubim. The changes promoted by herbicide exposure may be potentially disturbing for fish survival in an aquaculture farm. The type of pesticide used for pest control in agriculture fields should be chosen carefully to avoid possible contamination of fish farms.

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