Changes in intermediate metabolism and oxidative balance parameters in sexually matured three-barbeled catfishes exposed to herbicides from rice crops (Roundup®, Primoleo® and Facet®)

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1. Introduction

Pesticides are agricultural chemicals used with the primary objective of killing certain forms of life. However, they are often lethal to non-target species as well. As a result of their use in anthropogenic activities, these compounds can become aquatic contaminants. In addition to their lethality, these compounds can affect several levels of physiological and morphological organization— including muscle, liver, endocrine glands, and other tissues and organs— in various species and biological communities (Albinati et al., 2009). Fanta et al. (2003) highlight the importance of assessing the physiology of organisms exposed to sublethal concentrations of pollutants as a means of furthering our understanding of the adaptability of these organisms in contaminated environments. The authors also stress that these adaptations may differ depending on the stage of the life cycle of each species. Santos and Martínez (2012) note that, upon contact with an organism, toxic agents may undergo biotransformation, a process that makes xenobiotic compounds less toxic and facilitates their excretion. Thus, physiological analyses (based on measurement of biochemical markers) can provide evidence of adaptive responses to the stress caused by exposure to agrochemicals, such as mobilization of substrates in an attempt to maintain homeostasis.

Fish tissues are considered particularly sensitive to environmental stressors, as they clearly suffer ill effects from exposure to these chemical compounds (Albinati et al., 2009; Persch et al., 2017). Useful biomarkers include glycogen, which has been widely studied, as changes in this energy store may represent biochemical adaptation (Samanta et al., 2014). Sancho et al. (1998) and Persch et al. (2017). Proteins are also an extremely important substrate in an attempt to maintain homeostasis. In fish, protein catabolism triggers energy production; thus, declines in protein levels may represent a compensatory response to the stress caused by herbicide exposure (Samanta et al., 2014). Sancho et al. (2000) note that, in fish, exposure to pesticides can lead to disturbances of osmoregulation as a result of abnormal protein uptake by tissues.

According to Ahmad et al. (2000), environmental pollutants,
including herbicides, cause oxidative stress in a wide range of aquatic organisms—such as fish—as a result of increased production of reactive oxygen species (ROS), which occurs even in the absence of xenobiotics, and that responses vary not only between species, but also between tissue types; this is attributed to differences in antioxidant mechanisms. A study by Persch et al. (2017) found differential response patterns between different organs (gills, liver, muscle, and kidneys) in juvenile (sexually immature) *Rhamdia quelen* exposed to different herbicides. Sies (1993) explains that oxidative stress can be defined as an imbalance between pro-oxidant and antioxidant substances. Damage may occur when levels of pro-oxidant substances increase or when antioxidant levels decrease; this is known as oxidative stress. Once produced, ROSs react with cellular macromolecules in an attempt to achieve stability through chemical bonds (Sies 1993), which often leads to cumulative injury in tissues and organs composed primarily of proteins, lipids, and nucleic acids (Lushchak and Bagnyukova, 2006).

Although oxidative balance is still the object of extensive discussion in this taxon, fish—as other aerobic organisms—are known to have an effective antioxidant system, which works to protect against and mitigate the damage caused by ROSs (Trenzado et al., 2006; Zhang et al., 2008). Taking these aspects into consideration, the present study was conducted in *Rhamdia quelen* Quoy and Gaimard, 1824, a hardy fish species with a range extending from Mexico to Argentina (Reis et al., 2003). In Brazil, *R. quelen* is perfectly adapted to the seasons and extreme temperature changes, as well as to variations in water pH, hardness, ammonia content, and dissolved oxygen, and is a generalist omnivore (Gomes et al., 2000). It has good commercial acceptability and farming potential both for sport fishing and as food, in view of its palatable flesh and excellent features for industrial processing (Barcellos et al., 2003).

The present work sought to assess possible alterations in markers of intermediary metabolism and oxidative status in *Rhamdia quelen* adults exposed to sublethal and realistic concentrations of the herbicides Roundup®, Primolo® and Facet®. We expect to detect abnormalities both in markers of intermediary metabolism (total protein, total lipid, triglycerides, and glycogen) and in oxidative status parameters (superoxide dismutase, catalase, and thiorbarbituric acid-reactive substances) that denote energy expenditure in response to oxidative stress. We also hypothesize that these changes will be less intense than those previously observed by Persch et al. (2017) in juvenile (i.e., sexually immature) *R. quelen* exposed to similar concentrations of the same herbicides, but kept at a different ambient temperature.

### 2. Materials and methods

#### 2.1. Experimental protocol

Specimens were acquired from a fish farm. All were allowed to acclimate for 7 days in natural conditions during the summer, with temperature of 29 ± 3 °C and a 14:10-h light/dark cycle. Animals were kept in tanks with constant aeration, salinity 0 psu, pH 6.7–7.2, safe from any predators, and fed once daily (*ad libitum*) with the same commercially available feed used at the fish farm. Carneiro and Mikos (2005) claimed in their study that standard growth of *R. quelen* can be achieved with once-daily feeding. The sexual maturity length of this species is 16.5 cm for males and 17.5 cm for females (Baldisserotto and Neto, 2004); as all specimens had a total length > 18 cm, all were considered recently matured adults fit for reproduction.

The 252 fish were randomly divided into 21 aquariums containing 50 L water each (1 fish/4L water; 12 animals per aquarium and 36 per experimental group). Each experimental group was composed of three replicates. After the acclimation period, but remaining under the same conditions used during this period, animals were exposed for 7 days to the chemicals of interest, at different sublethal concentrations, similar to those found in natural settings. Animals were allocated across seven experimental groups for this study: a control group, which was not exposed to any agricultural chemicals throughout the 14-day experiment period; the Roundup® (18 μg/L and 72 μg/L) groups; the Primolo® (10 μg/L and 15 μg/L) groups; and the Facet® groups (1.75 μg/L and 14 μg/L).

The three tested herbicides are widely used on several crops grown in Southern Brazil, especially in rice paddies. Roundup® (glyphosate, manufactured by Monsanto do Brasil Ltda.) is used for a wide range of applications in agriculture. According to Amarante et al. (2002), the recommended dose is approximately 5 L/ha, applied up to four times at 20- to 40-day intervals. Primolo® (atrazine, manufactured by Syngenta Proteção de Cultivos Ltda.) is indicated for broadleaf weed control in grain crops (Griboff et al., 2014), including maize, rice, wheat, sorghum, barley, and sugarcane. Bortoluzzi et al. (2006) found atrazine concentrations of 0.20 and 0.63 μg/L in surface water samples after tobacco seedling transplant. In Brazil, use of these two herbicides is regulated by National Environment Council (CONAMA) Resolution No. 357. The permissible concentrations of glyphosate and atrazine in natural environments are up to 500 μg/L and 2 μg/L respectively. There is still no legal regulatory framework for Facet® (quinclorac, manufactured by Basf S/A) in Brazil. Quinclorac belongs to a new class of highly selective herbicides known as auxin herbicides or auxin mimetics. It is also indicated for use in grain crops and other grasses, and is marketed as a highly soluble powder (Toni et al., 2013). Marchesan et al. (2007) found traces of quinclorac when monitoring rice paddies in Rio Grande do Sul; concentrations from 0.48 to 6.60 μg/L were detected in the Vacacai-Mirim River.

According to Brazilian Ministry of Health Ordinance No. 518/2004, the maximum permissible concentration of glyphosate in drinking water destined for human consumption is 0.5 mg/L. In the United States, the Environmental Protection Agency (EPA)-defined limit for drinking water is 700 μg/L. Queiroz et al. (2011) reported detection of glyphosate concentrations ranging from 0.1 to 0.7 mg/L in surface waters sampled in soybean-growing areas. Therefore, our experiments were designed with Roundup® concentrations of 18 and 72 μg/L, both of which are within the range detected in natural environments and within the range permitted by current Brazilian legislation.

The permissible concentration of atrazine, the active ingredient of Primolo®, in drinking water destined for human consumption is 2 μg/L (according to Brazilian Ministry of Health Ordinance No. 518/2004). In the United States, the EPA-defined limit for this herbicide in bodies of water is 3 μg/L. Based on the findings of Paulino et al. (2012), who detected atrazine concentrations in the range of 0.2–1,000 μg/L in bodies of water adjacent to treated areas, we designed our experiments using 10 μg/L and 15 μg/L concentrations of Primolo®.

To support our choice of concentrations for Facet®, we used as a reference the work of Deschamps et al. (2013), who found quinclorac concentrations in the range of 2.11–20.2 μg/L in freshwater rice paddies in the southern region of the state of Santa Catarina, Brazil. Thus, we selected the Facet® concentrations of 1.75 and 14 μg/L. The higher concentration is within the range of values detected by the authors, while the lower concentration is not. This decision was made because we sought to assess whether biological effects occur even with exposure to extremely low concentrations of Facet® that are considered sublethal and safe for non-target organisms, as there are no regulations for quinclorac use in Brazil.

After the acclimation and exposure periods, the specimens were killed by brainstem/spinal cord transection, weighed on a semi-analytical balance (precision 0.1 g), and measured with a digital caliper (resolution 0.01 cm). The gills, liver, kidneys, and part of the tail muscle (approximately 1 cm²) were resected from animals of both sexes (males and females). Due to the discrepancy in number between males and females, tissues were not separated by sex for analysis. Tissues were then frozen at −20 °C and processed as appropriate for biochemical analyses. The study protocol was approved by the PUCRS Institutional Animal Care and Use Committee, with registration no. 11/00276.
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