



Metabolic parameters and oxidative balance in juvenile *Rhamdia quelen* exposed to rice paddy herbicides: Roundup[®], Primoleo[®], and Facet[®]



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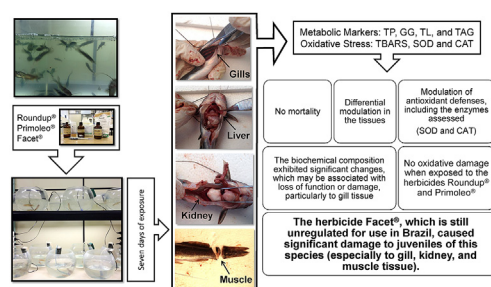
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HIGHLIGHTS

- Fish showed no mortality when exposed to realistic concentrations of these herbicides.
- Differential changes in biochemical composition were observed in the fish exposed to different herbicides.
- *R. quelen* specimens appear to be capable of modulating their antioxidant defenses.
- This species sustains no oxidative damage when exposed to the herbicides Roundup[®] and Primoleo[®].
- Facet[®] caused significant damage to juveniles of this species.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study sought to assess the response of *Rhamdia quelen* juveniles (6–8 cm total body length) to exposure to different concentrations of three herbicides: Roundup[®] Original (18, 36, 72, and 144 µg/L), Primoleo[®] (2.5, 5, 10, and 15 µg/L), and Facet[®] (1.75, 3.5, 7, and 14 µg/L). Total protein (TP), glycogen (GG), total lipids (TL), triacylglycerols (TAG), lipid peroxidation (TBARS), and activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in gills, liver, kidneys, and muscle were measured by spectrophotometry. Roundup[®] (glyphosate) reduced the TP, GG, and TL in gills and TL in liver and kidney and increased TP in liver and increased GG in muscle. In contrast to Primoleo[®] (atrazine), all tissues stored TAG and consumed LT, besides the gills also reduced PT. There was still an increase in GG in the kidneys and muscle. Facet[®] (quinclorac) induced changes mainly in the liver (increased TP, TL, and TAG content) and muscle (increased GG, TL, and TAG depletion). Gill tissue exhibited TP depletion alone, and kidney tissue metabolism was unchanged. This fish species appears capable of modulating its enzymes to the point where it sustains no oxidative damage as a result of exposure to the herbicides glyphosate (possibly due to increased CAT activity), atrazine (despite no changes in SOD or CAT activity), and quinclorac (with increased lipid peroxidation, particularly in gill, kidney, and muscle tissue, despite elevated SOD activity). Although it is not considered a target species, *R. quelen* suffers harmful effects from interaction with these herbicides.

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1. Introduction

Fish are excellent biomarkers of environmental degradation, as they are sensitive to a wide range of stressors and their successful reproduction, growth, and population survival are highly dependent on water quality and on some species of macroinvertebrates as a food source (Fausch et al., 1990). Given their long lifespan, fish show effects such as reproductive failure and mortality at different age classes as consequences of environmental stressors. Thus, the greatest advantage of using fish as indicator organisms is their ability to demonstrate the quality of an aquatic ecosystem and the surrounding watershed, as they manifest the ecological significance of direct and indirect stresses on the ecosystem (Fausch et al., 1990). Menezes et al. (2014a) advocate the use of Teleostei as indicators of contamination by pollutants (such as pesticides), as their biochemical responses are similar to those found in mammals.

Rhamdia quelen Quoy and Gaimard, 1824, the Jundiá, is a species of catfish (class Osteichthyes, infraclass Teleostei, order Siluriformes, family Heptapteridae). Its distribution ranges from Mexico to Argentina (Reis et al., 2003) and it is considered a hardy species, due to its ability to survive changes in water parameters such as pH, hardness, ammonia, and oxygen content (Gomes et al., 2000). Its color ranges from pale reddish brown to gray, with a lighter ventral area. This color varies in accordance with the environment; specimens tend to be paler in brighter environments and darker in dim environments. *R. quelen* is a three-barbeled catfish, i.e., it has three pairs of barbels near its mouth. These appendages probably aid in perception of water quality and in the search for food (Baldiasserotto and Neto, 2004). In its natural habitat, this species is a generalist omnivore, exhibiting a preference for fish, crustaceans, insects, vegetable debris, and organic detritus, which reflects the fact that it also feeds outside the benthic zone (Gomes et al., 2000). In Brazil, this species is perfectly adapted to the seasons and extreme temperature changes; it is able to survive the low temperatures of winter and achieve successful growth and reproduction in the summer. Other vernacular names include *jandiá*, *mandi*, and *sapi-poca*. 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).

Agricultural chemicals affect and pollute aquatic environments, which these compounds reach through several routes, including direct application, industrial and urban wastewater, runoff from diffuse sources (including agricultural soils), aerosols, particulate deposition, and precipitation (Ballesteros et al., 2009; Sharma, 1990). Growing concern with man-made changes to the aquatic environment has given rise to a need for standardization and more widespread use of indicator organisms, as well as the development of toxicity tests or bioassays to assess the impact of these changes. Monitoring the degree of environmental degradation and the health of lake and river ecosystems mandates the use of fish as indicator organisms (Hued and Bistoni, 2005). The method of detecting abundance of tolerant species and disappearance of intolerant taxa has been widely used to indicate and measure environmental degradation (Fausch et al., 1990).

According to Fanta et al. (2003), the importance of physiological evaluations in organisms exposed to sublethal concentrations lies in the fact that these evaluations provide an understanding of the capacity of populations to sustain themselves in contaminated environments. The authors also found that many pesticides are ineffective due to their low toxicity, but are then metabolized into toxic active compounds. Biochemical markers can provide information on the pesticide metabolism process; according to Santos and Martinez (2012), toxic agents

often undergo enzyme-mediated biotransformation into less toxic compounds, which facilitates their excretion. Studies have shown that herbicides can disrupt carbohydrate and protein metabolism, as well as several blood parameters, in some fish species (Pereira et al., 2013; Menezes et al., 2014a,b).

To Shiogiri et al. (2012), gills and liver are the main target organs for xenobiotics in fish. In the liver, disturbances in lipid and carbohydrate metabolism may occur, indirectly affecting the function of other organs. Paulino et al. (2012a) noted that gills, kidneys, and intestines play an important role in biotransformation and excretion of agrochemicals, and the liver is known to be the main site of these processes. These authors concluded that metabolic and enzymatic responses act as protective mechanisms against absorption of toxic substances, but if such environmental conditions persist long enough, tissue damage (particularly to the gills) may jeopardize overall metabolism, including the pathways involved in tissue repair, growth, and reproduction.

According to Costantini (2014), oxidative stress is characterized by a decline in antioxidant defenses, formation of reactive nitrogen species (RNS) or reactive oxygen species (ROS), or both. ROSs include the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\bullet}OH$). This scenario is caused by an imbalance between formation of pro-oxidant and antioxidant compounds. Enzymatic antioxidant defenses include superoxide dismutase (SOD), which catalyzes conversion of O_2^- into H_2O_2 ; catalase (CAT), which catalyzes the breakdown of H_2O_2 into O_2 and H_2O ; and glutathione peroxidase, which catalyzes the decomposition of non- H_2O_2 organic peroxides in general (Thomaz et al., 2009; Costantini, 2014). These ROSs can react with biological macromolecules to induce lipid peroxidation (LPO), DNA damage, and protein oxidation, potentially leading to oxidative stress. Thomaz et al. (2009) and Lushchak (2016) found that different pathological processes involved in the etiology of several fish diseases are associated with contaminants that trigger excessive stimulation of ROS production. To the authors, these conditions occur in several taxa present in aquatic ecosystems. Tropical ecosystems in particular are currently threatened by environmental degradation and anthropogenic processes, including indiscriminate use of agrochemicals (insecticides, pesticides, and herbicides).

The exact formulation of glyphosate-based herbicides varies widely across manufacturers. The most popular formulation consists of the isopropylamine (IPA) salt of *N*-(phosphonomethyl) glycine, surfactant, and water. Commercial formulations containing polyoxyethylene amine (POEA) as surfactant have a half-life of up to 197 days in soil and up to 42 days in water (Giesy et al., 2000). In Brazil, glyphosate is manufactured and marketed by Monsanto do Brasil Ltda. as Roundup®, the same trade name used in North America. In other countries, however, this herbicide is available under several other names, such as Sting, Alpheo, Azural, and Faena. Glyphosate is an inhibitor of shikimic acid, a compound that occurs exclusively in plants and has no equivalent in animals. As there is no enzyme with the potential to be affected, this compound causes damage to several organs when ingested by aquatic animals (Aminov et al., 2014). The same authors found that aquatic animals, particularly fish, are more sensitive than mammals to Roundup®. Several authors have conducted studies on the effects of Roundup® in different fish species: Peppered coridora *Corydoras paleatus* by Fanta et al. (2003); Jundiá *Rhamdia quelen* by Ferreira et al. (2010), Menezes et al. (2011), and Soso et al. (2007); Piavçu *Leporinus macrocephalus* by Albinati et al. (2009); and Nile tilapia *Oreochromis niloticus*, Common carp *Cyprinus carpio*, and Tambaqui *Colossoma macropomum* by Moura (2009). Despite different results and distinct changes dependent on the experimental procedures, all

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