Environmental Science and Pollution Research (2021) 28:4156–4172
https://doi.org/10.1007/s11356-020-10654-x

RESEARCH ARTICLE

Melanophryniscus admirabilis tadpoles' responses to sulfentrazone and glyphosate-based herbicides: an approach on metabolism and antioxidant defenses

Patrícia Rodrigues da Silva[1] · Márcio Borges-Martins[2] · Guendalina Turcato Oliveira[1]

Received: 18 December 2019 / Accepted: 26 August 2020 / Published online: 15 September 2020

Abstract

Melanophryniscus admirabilis is a frog endemic to the southern Atlantic Forest (Brazil), with restricted distribution and considered as critically endangered. The aim of this study was to evaluate possible alterations in biomarkers of metabolism (glycogen, proteins, and uric acid) and oxidative balance (superoxide dismutase, catalase, glutathione S-transferase, and lipoperoxidation) of tadpoles of *Melanophryniscus admirabilis* exposed to commercial herbicide formulations containing sulfentrazone (Boral® 500 SC: 130 and 980 μg a.i./L) and glyphosate (Roundup® Original: 234 and 2340 μg a.i./L). Mortality was not observed in any of the groups studied. Our results show that a 96-h exposure to the herbicides decreased glycogen levels, indicating increased energy demand for xenobiotic metabolism. Protein levels increased in the Boral group but decreased in the higher concentration of Roundup, and uric acid levels did not change significantly between the experimental groups. Lipoperoxidation decreased in the Boral group and in the higher concentration of Roundup. Decreased levels of superoxide dismutase in both treatments and of catalase in the lowest concentration of the herbicides were observed. Glutathione S-transferase activity increased in the Roundup group; this enzyme seems to be crucial in the metabolization of the herbicides and in the survival of the tadpoles. Our results suggest that *M. admirabilis* has a high antioxidant capacity, which guaranteed the survival of tadpoles. Nevertheless, exposure to pesticides could impose a serious risk to this species, especially considering its restricted distribution, habitat specificity, and high physiological demand to metabolize xenobiotics.

Keywords Amphibians · Biomarkers · Pesticides · Intermediate metabolism · Oxidative balance

Responsible Editor: Philippe Garrigues

Highlights

- Tadpoles were exposed to commercial herbicide formulations containing sulfentrazone and glyphosate.
- Mortality was not observed.
- Glycogen has been mobilized as an energy substrate for the detoxification process.
- Only uric acid levels and catalase activity did not vary significantly.
- No increase in lipid peroxidation was observed in any of the herbicides.
- GST increases at all herbicide concentrations.

✉ Guendalina Turcato Oliveira
guendato@pucrs.br

[1] Laboratório de Fisiologia da Conservação, Departamento de Ciências Morfológicas, Programa de Pós-Graduação em Ecologia e Evolução da Biodiversidade, Escola de Ciências da Saúde e da Vida, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, Brazil

[2] Laboratório de Herpetologia, Departamento de Zoologia, Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

Introduction

Brazil presents greatest amphibian richness in the world, with approximately 1100 species, corresponding to nearly 14% of the 8121 species known worldwide (AmphibiaWeb 2020). More than half of the amphibian fauna from Brazil were described in the last 60 years, and knowledge of most species is still insufficient (IUCN 2020; Segalla et al. 2016). At the federal level, 41 species are officially recognized as threatened and 22 are near threatened, and for 167, the data required to determine their status are deficient (ICMBio 2018). Ecotoxicological studies carried out with pesticides have normally considered generalist species which are widely distributed in the northern hemisphere. Rich tropical regions have been critically underrepresented in these studies and, consequently, current knowledge can considerably underestimate the sensitivity of tropical and subtropical amphibians to pollutants (Schiesari et al. 2007).

Taking into account the economic importance of agriculture in Brazil, the country has been the largest agrochemical

consumer worldwide since 2008, representing approximately 20% of total world agrochemical use (Albuquerque et al. 2016), and nearly 50% of the agricultural pesticides are consumed in Latin America (ANVISA 2005). The average agrochemical consumption in the country is one million tons per year, representing 5.2 kg per resident. In the last decade, the agrochemical market grew 190% in the country, comprised mainly of herbicides (SINDAG 2011; Walker 2014).

In the last decades, amphibian populations have declined worldwide, and the explanation may be related to the synergistic effects of several different factors. Habitat degradation and fragmentation, climatic changes—including global warming and an increase in UV-B radiation, emerging infectious diseases (especially epidemics caused by the fungus *Batrachochytrium dendrobatidis*), and aquatic and terrestrial pollution, notably related to the increased use of pesticides in agriculture, are the main factors related to amphibian declines (Collins and Storfer 2003). Pesticides can leach into places far from the location of their application due to their solubility and mobility in different substrates, thus reaching non-target organisms (Peres and Moreira 2003). Although these pesticides were designed to act only on specific target organisms, several studies have demonstrated physiological changes in non-target organisms, such as amphibians (Coltro et al. 2017; Dornelles and Oliveira 2014, 2016; Wilkens et al. 2019). As a result of this set of biochemical–functional changes, pesticides can also affect the survival and reproduction of non-target organisms, including amphibians (Bókony et al. 2017; Gill et al. 2018). According to Relyea (2005), fungicides and herbicides are the pesticides with the greatest potential to cause changes in natural aquatic communities.

Sulfentrazone (N-[2,4-dichloro-5-(4-difluoromethyl)-4,5-dihydro-3methyl-5-oxo-1H-1,2,4-triazol-1-il]phenyl]methanesulfonamide) is the active ingredient of Boral® 500 SC. This herbicide belongs to the aryl triazolinone chemical group and is considered a potential contaminant due to its long persistence in soil (estimated half-life of 110–280 days) and relatively high-water solubility (Melo et al. 2010). Furthermore, this herbicide also has a high surface runoff and a high potential to leach into groundwater (Miller 1997). The solubility in water changes with the pH, being 110, 780, and 1600 mg L^{-1} at pH 6.0, 7.0, and 7.5, respectively. Little is known about the transport and destination of sulfentrazone in the environment; however, its first dissipation pathway includes microbial degradation (Melo et al. 2010).

Sulfentrazone concentrations in rivers, lakes, lagoons, and temporary waters in Brazil are still unknown; however, evidence shows the presence of sulfentrazone in agricultural areas in São Paulo (Santos et al. 2015). Dutra de Armas et al. (2005) carried out a survey of the amount of sulfentrazone used in the Corumbataí river basin, in the state of São Paulo, Brazil, and related the amount used in the region to the GUS coefficient and the LEACH index of the herbicide. Thus, noting that sulfentrazone is a potential contaminant of water sources in the region. Through water sampling in springs and artesian wells in the Córrego-Rico region, in Jaboticabal, São Paulo, Brazil, Santos et al. (2015) detected sulfentrazone residues with a concentration of up to 0.6 ppb in 15.4–30% of springs and 21.9–34.4% in artesian wells, varying according to the sampling time. Gehrke et al. (2020) in its review on sulfentrazone presents an overview of the use of this herbicide, advantages and disadvantages of its use in Brazil, in addition to its potential as an environmental contaminant. Concentrations of the herbicide's atrazine and sulfentrazone were measured in the soil, runoff water, and groundwater of rotational fields of corn and soybeans in Illinois (USA) (Thorngren et al. 2017). These authors found a maximum concentration of sulfentrazone in the runoff water of 25.3 μg/L, with an average concentration of 10.3 μg/L (ranging from 0.056 to 25.3 μg/L), and the mean concentration of sulfentrazone in groundwater was 7.5 μg/L (ranging from 2.5 to 21.6 μg/L).

There are few studies on the effects of sulfentrazone in nontarget aquatic species other than those conducted by the US Environmental Protection Agency (USEPA) for sulfentrazone, and these studies indicate that nontarget aquatic vascular plants are the most susceptible to sulfentrazone, followed by phytoplankton, aquatic invertebrates, and fish (Thorngren et al. 2017). Sulfentrazone can have negative effects on amphibians, as evidenced by Freitas et al. (2017) where the herbicide caused the disruption of the oxidative balance of two species of amphibians native to Brazil (*Rhinella schneideri* and *Physalaemus nattereri*). Wilkens et al. (2019) also observed changes in the energy reserves, plasma corticosterone, and oxidative balance of bullfrog tadpoles (*Rana catesbeiana*) exposed to Boral® 500SC.

Roundup® Original is one of the commercial formulations of the active ingredient glyphosate (N-(phosphomethyl) glycine). Glyphosate-based herbicides are one of the most frequently used pesticides in agriculture activities around the world because they have a broad action spectrum (Grube et al. 2011). Annett et al. (2014) in their review of the impact of glyphosate and glyphosate-based herbicides on the freshwater environment report a half-life of 7–142 days in water, and Montgomery et al. (2008) report an average time spent in water between 7 and 70 days for glyphosate. Glyphosate can penetrate water bodies directly via spray application or indirectly through leaching after periods of rain following its use in plantations (Botta et al. 2009).

In South America, mainly Brazil and Argentina, glyphosate is applied several times during the year (Beckie et al. 2020). In a study of surface water carried out in the Pampas of Argentina, glyphosate concentrations were found in the order of 100 to 700 μg/L (level of detection of 0.04 mg/L) (Peruzzo et al. 2008). Pérez et al. (2011) report that in Canada and the USA the highest concentrations of glyphosate were

found in a natural environment (bodies of water), these being 1.24 mg a.e./L (Newton et al. 1994), 1.54 mg a.e./L (Couture et al. 1995), 2.8 mg a.e./L (Legris and Couture 1989), and 5.2 mg a.e./L (Edwards et al. 1980). According to the review by Annett et al. (2014), the levels of glyphosate found in surface waters of different regions in the world are quite variable (< 0.01–1.95 mg/L). Moutinho et al. (2020) report that there are about 31 formulations containing glyphosate as an active ingredient in Brazil, the maximum recommended dose for this herbicide is 2400 g ai/ha for sugarcane, which is like soy. These same authors estimated that a puddle of 10 m^2 in area and 10 cm in depth (therefore 1000 L of volume) sprayed with glyphosate at 2400 g ai/ha would receive 2.4 g ai, reaching a concentration of 2.4 mg of a.i./L or 2400 μg a.i./L.

Several studies have reported the negative effects of glyphosate and its formulations on different organisms, especially aquatic ones (e.g., Avigliano et al. 2014; Gill et al. 2018; Govindarajulu 2008; Persch et al. 2017, 2018; Wagner et al. 2013). These effects include alterations in metabolism, oxidative balance, development, growth, genetic parameters, and the survival of the tested animals. According to Annett et al. (2014) the surfactants used to increase herbicide efficacy have been identified in some studies as the chemicals responsible for toxicity of glyphosate-based herbicides to non-target species, yet they are often difficult to chemically identify. It is important to note that the commercial formulation of herbicides is a mixture of various ingredients, but the exact nature and quantity of chemicals added to the active ingredient are often undisclosed due to trade secret, which makes it difficult to assess their behavior and physical-chemical characteristics or toxicity. Thus, toxicological tests with commercial formulation need to be carried out.

Changes in metabolic and physiological parameters are among the consequences to tadpoles of exposure to pesticides. Glycogen, lipids, and triglycerides act as precursors of ATP synthesis and are mobilized in energy release, especially during stressful situations, such as in polluted environments (Coltro et al. 2017; Dornelles and Oliveira 2014, 2016; Wilkens et al. 2019). Proteins might also be involved in compensatory mechanisms in polluted conditions, acting as precursors of ATP synthesis in the generation of energy (Adamu and Kori-Siakpere 2011). However, processes of protein catabolism may lead to cellular and tissue disruption and eventual death since those metabolites have structural roles (Coltro et al. 2017).

Oxidative balance biomarkers have been widely used to evaluate the effects of pesticides on amphibians (Gripp et al. 2017; Zhang et al. 2019). During ATP synthesis through oxidative pathways, a small percentage of oxygen (2–5%) consumed by mitochondria is converted into reactive oxygen species (ROS) (Costantini 2014). ROS are pro-oxidant components with an important biological role; however, when in excess, they may cause damage to organic molecules and create a situation known as oxidative stress (Costantini 2014; Schier and Chandel 2014). Oxidative stress results from the unbalance between the formation and removal of pro-oxidant agents by the organism's antioxidant system leading to damage of molecules, such as DNA, RNA, proteins, carbohydrates, and lipids (Halliwell and Gutteridge 2007). The lipid peroxidation process (LPO) is an oxidative chain reaction resultant from the action of ROS on the phospholipids of cell membranes. This process is considered one of the best predictors of the intensity of systemic damage induced by pro-oxidants (Ojha et al. 2011).

To avoid or prevent oxidative damage caused by pro-oxidant agents during the cell metabolism, aerobic organisms developed an antioxidant system composed of a non-enzymatic and an enzymatic component (Costantini 2014). Non-enzymatic antioxidants include low molecular weight molecules, such as glutathione, uric acid, melatonin, ascorbic acid, and tocopherol, among others (Coltro et al. 2017; Hermes-Lima 2004; Tsahar et al. 2006). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are the most important and best-known antioxidant enzymes responsible for neutralizing the action of ROS in biological systems. SOD catalyzes the dismutation of the superoxide anion into oxygen and hydrogen peroxide, while CAT and GPx work coordinately to eliminate hydrogen peroxide and organic peroxides from the organism (Costantini 2014).

Exposure to xenobiotics may be associated with increased expression and/or activity of biotransformation enzymes, including glutathione S-transferases (Hermes-Lima and Storey 1993; Taysse et al. 1998). The biotransformation process of organic compounds can be divided into the following three phases: phase I, where oxidation/hydroxylation reactions of the molecules occur; phase II, where there is conjugation of xenobiotics with endogenous components, such as glutathione in a reaction catalyzed by GST; and phase III, related to the excretion of xenobiotics (van der Oost et al. 2003; Nunes et al. 2011).

Among the fitness components, which can be affected by pollutants are energy reserves and oxidative balance. Trade-offs or the allocation of energy between growth, reproduction and survival mechanisms have been recognized as an important mediator in the life history of organisms. The oxidative balance and the consequent management of oxidative stress are likely to be determinant not only for life history, but also for the performance of organisms which use oxidative pathways for ATP production (Costantini 2014; Monaghan et al. 2009).

Oxidative stress results from a mismatch between the production of damaging reactive oxygen species (ROS) and the organism's capacity to mitigate their damaging effects. The levels of oxidative stress incurred by organisms are not constant but vary with the stage of development, environmental conditions, and levels of activity. It is known that in different organisms, ROS are involved in different animal physiological processes constituting an important factor in the modulation of the immune system (defense against pathogens), in the

modulation of the endocrine system and in the oxidation of xenobiotic chemical agents (Dowling and Simmons 2009; Costantini 2014). In this sense, oxidative stress can be divided into (1) physiological or eustress (low levels), which is important in signaling and redox regulation, while overload or (2) distress (high or supraphysiological levels) would lead to the interruption of redox signaling and/or oxidative damage to biomolecules (Sies 2018).

Melanophryniscus admirabilis (Bufonidae) is a small toad, critically endangered (IUCN 2013; ICMBio 2018) with a microendemic distribution and a population which occurs exclusively in an area of 700 m along the rocky banks of the Forqueta River, in a forest with steep slopes in the southern region of the Brazilian Atlantic Forest (Di-Bernardo et al. 2006; Fonte et al. 2014). However, the species abundance is relatively high. Vasconcellos (2015) observed that abundance of adult individuals in the reproductive site varied from 14 (non-reproductive season) to 929 (reproductive season), with an estimate of discrete and stable population growth ($\lambda = 1.04$).

The study area, known as Perau de Janeiro in Arvorezinha (Brasil, RS), is a fragment of mixed tropical forest that has been in regeneration for at least 30 years, according to a report of abandonment of agricultural activities previously developed in the area. According to the more recent Brazilian threatened fauna red book, a potential threat still unquantified is constituted by pesticide contamination from tobacco crops adjacent to slopes of the Forqueta River valley (ICMBio 2018). Furthermore, studying the impact of pesticides on habitat and populations of *M. admirabilis* represents a priority, which is included in the National Action Plan for Conservation of Amphibians and Endangered Reptiles in Southern Brazil (ICMBio 2012). In this region, we find increase in agricultural activity related to the cultivation of yerba mate, corn, tobacco, soybean, and planted forests (silviculture: *Eucalyptus* sp. and acacia); areas which have been expanding in recent years (EMATER 2001).

In this sense, to provide required information demanded by conservationists and environmental agencies, this study aimed to evaluate possible alterations in the metabolic profile (glycogen, proteins, and uric acid) and oxidative balance (superoxide dismutase, catalase glutathione S-transferase, and lipoperoxidation levels) of *M. admirabilis* tadpoles exposed to different concentrations of Boral® 500 SC (sulfentrazone) and Roundup® Original (glyphosate). These herbicides, in this commercial formulation, are widely used in the region of the state where *M. admirabilis* occurs.

Methods

Tadpole sampling

The study was authorized by the Committee on Animal Research and Ethics from the Pontifícia Universidade Católica do Rio Grande do Sul (CEUA/PUCRS) (Permit n° 6879). Sampling size and procedures were authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBio) (Permit n° 40004-4) and followed the legal precepts of the Federative Republic of Brazil. A total of 90 *Melanophryniscus admirabilis* tadpoles were sampled from natural pools in Arvorezinha municipality (28°51′28″ S, 52°18′12″ W), State of Rio Grande do Sul, Brazil. The tadpoles corresponded to stages 25–30 according to Gosner's staging table (Gosner 1960; Santos et al. 2016b). After sampling, the animals were kept in plastic containers with water from the sample site and constant aeration and transported to the Laboratório de Fisiologia da Conservação (PUCRS), where the experiments were performed.

Acclimation and experimental design

In the laboratory, the tadpoles were bathed in cold water (8–10 °C) to reduce their metabolism and stress levels. Afterwards, they were weighed on an electronic scale (0.001 g precision) and the rostrocaudal length was measured with a digital caliper (0.01 cm precision). Tadpoles of comparable body weight were randomly distributed in glass aquaria, with a maximum capacity of 5 L, filled with dechlorinated water. In all treatments, the tadpole density of one individual per 1 l of water was maintained. Throughout the experimental period (nine days: five-day acclimatization and four-day herbicide exposure), the aquariums were kept constantly aerated without substrate. The animals were fed ad libitum once daily with commercial fish feed containing 22.5% crude protein with the quantity of food being approximately 5% of the biomass contained in each aquarium. The room temperature was set to 23 ± 1 °C and to a 12 h light:12 h dark photoperiod.

The animals were acclimated to these conditions for five days; at day 6, herbicides were added to the exposed experimental groups; they remained for another four days under these conditions. After the acclimation period, 15 animals (defined as the acclimation group) were stunned by a cold-water bath (4 °C), weighed, and measured. The tadpoles were immediately frozen in liquid nitrogen (CONCEA 2013) and kept frozen at − 20 °C to maintain body integrity until biochemical analyses were carried out (Coltro et al. 2017; Wilkens et al. 2019). The remaining animals (75) were divided into the control group maintained in dechlorinated water without herbicides, and groups exposed to two different concentrations of commercial formulation of sulfentrazone (Boral® 500 SC: B-130 μg a.i. sulfentrazone/L and B-980 μg a.i. sulfentrazone/L) and two others of glyphosate (Roundup® Original: R-234 μg a.i. glyphosate/L and R-2340 μg a.i. glyphosate/L) (a.i. active ingredients). Each group had three replicates with each replicate held in a separate 5 L aquarium with five tadpoles in each, totalizing 15 animals per experimental group.

Abadie and Silva (pers. obs.) estimated that *M. admirabilis* completes the metamorphic process within 15 days. The experimental period was settled in a way that the animals would not complete the metamorphosis during the experiments. Some animals presented budding and/or exteriorization of the hind limbs (stages 31–36 of Gosner) at the end of the acclimatization period; they were evenly distributed, from one to two animals per experimental group. In general, the developmental stages of animals in each of the experimental groups were the following: (1) initial, animals between stages 25 and 30; (2) acclimation, animals between 31 and 36; (3) exposure control; (4) B-130 μg a.i./L; (5) B-980 μg a.i./L; (6) R-234 μg a.i./L; and (7) R-2340 μg a.i./L, animals between 34 and 39 of Gosner.

After the herbicide exposure period, all tadpoles were weighed, measured, and euthanized by instantaneous freezing in liquid nitrogen, as described previously. The pH, ammonia content (ppm), dissolved oxygen (mg/L), conductivity (μs/cm), and oxidation reduction potential (mV) were measured twice daily, throughout the experiment period in each aquarium with a multiparametric probe.

Herbicides

The agrochemicals were diluted with distilled water to formulate a stock solution of Boral® 550 SC (product of FMC, composed of Sulfentrazone 50% *w*/*v* plus other ingredients 72.16% w/v, registered at the Ministry of Agriculture, Livestock and Supply, No. 07495) (1 g/L) and Roundup® Original (product of Monsanto, containing isopropylamine salt of N-phosphonomethylglycine 48.0% *w*/*v*, plus acid equivalent of N-phosphonomethylglycine 36.0% w/v, and inert ingredients 68.4% w/v, registered at the Ministry of Agriculture, Livestock and Supply, No. 898793) (1 g of active ingredient/L), from which aliquots were removed and mixed with aquarium water to obtain study concentrations.

Commercial glyphosate-based herbicides contain other components, which are called inert ingredients. These inert ingredients are mainly surfactants, solvents, and antifoam compounds. The surfactant in Roundup®, as well as in some other glyphosate-based products, is the highly toxic polyethoxylated tallowamine compound (Pérez et al. 2011). This material is referred to in the literature as MON0818, or polyoxyethyleneamine (POEA), present at about 15% in Roundup®. Numerous contributions have demonstrated that inert ingredients in glyphosate formulations have several folds higher toxicity on non-target organisms than glyphosate alone (Pérez et al. 2011). Therefore, glyphosate formulations are chemical mixtures and must be considered as mixtures in toxicity assessments.

The concentrations of herbicides used in our experiments (Boral® 500 SC: B-130 μg a.i./L and B-980 μg a.i./L; Roundup® Original: R-234 μg a.i./L and R-2340 μg a.i./L) were determined nominally, without chemical confirmation. There is little information about the sulfentrazone concentration in surface waters of Brazilian rivers. Thus, the sulfentrazone concentrations chosen were based on bioassays made by Thorngren et al. (2017) with larvae of the fish *Pimephales promelas* exposed to sulfentrazone between 230 and 968 μg/L. The glyphosate concentrations chosen are within the concentration range used in agriculture in Brazil, which varies from 0.36 to 2.16 mg/L, according to Rodrigues and de Almeida (1998). Considering the half-life of each of these herbicides (between 110 and 280 days for sulfentrazone and between 7 and 70 days for glyphosate), it is unlikely that the concentrations used underwent any significant decay in the aquariums during the exposure period (4 days). In subsequent experiments on *M. admirabilis* tadpole exposure to glyphosate, the PUCRS Environmental Analytical Chemistry Laboratory quantified and confirmed the non-decay of this molecule in the water and in a glyphosate stock solution (1 g a.i./mL) prepared from Roundup® Original (unpublished data).

Biochemical analyses

Intermediate metabolism parameters

To determine the biomarkers of intermediate metabolism of the tadpoles, three pools consisting of two animals each were prepared. The pools were used for the quantification of glycogen, proteins, and uric acid levels. Each parameter being quantified in five replicates, of each pool, by spectrophotometry (CARY 3E-UV-Visible Spectrophotometer VARIAN); data were expressed as μg/g of wet weight^{-1}.

Glycogen was extracted according to Van Handel (1965) and estimated as glucose using the Biotécnica Kit based on the oxidase glucose method. The total protein and uric acid levels were estimated in aliquots of the medium obtained for the glycogen extraction using the Biotécnica Kit specific for these metabolites.

Oxidative balance parameters

To determine the parameters of oxidative balance, three pools consisting of three animals each were prepared. An aliquot of 5 mL of a solution composed of phosphate buffer (20 mM), potassium chloride (140 mM), and phenyl methyl sulfonyl fluoride (a protease inhibitor) (1 mM) was added for each gram of the tadpole body weight in each pool. Each pool was homogenized with an Ultra-Turrax (IKA-WERK) at 0–4 °C. The homogenized solution was centrifuged at 4 °C for 10 min at 1000 ×*g* using a refrigerated centrifuge (SORVALL RC-5B). The precipitate was discarded, and the supernatant fluid was removed, fractionated into four Eppendorf tubes, and frozen at − 20 °C to be used for further measurements

(Llesuy et al. 1985). All estimates were performed by spectrophotometry based on at least five replicates from each pool in the experimental group.

The LPO levels were estimated according to Buege and Aust's (1978) method, in which the biological material is heated to 100 °C in the presence of thiobarbituric acid (TBA). The condensation of substances reactive to TBA creates products visible at 535 nm (Lima and Abdalla 2001), namely thiobarbituric acid reactive substances (TBARS). The TBARS concentration was expressed as μmol TBARS/mg of protein.

The technique for estimating the SOD activity was based on the method described by Boveris and Cadenas (1982). This method consists of inhibiting the reaction of the superoxide radical with adrenaline by quantification in relative units. A unit of SOD is defined as the amount of enzyme that inhibits the rate of reduction of the detector (adrenaline) by 50%. The estimation of SOD activity was expressed as SOD U/mg of protein.

To estimate the CAT activity, the method described by Boveris and Chance (1973) was used with the previous activation of the enzymatic system according to Galbraith et al. (1983) and Endemann et al. (2001). The CAT activity levels were expressed as pmol CAT/mg of protein min.

The GST activity was estimated according to the method described by Boyland and Chasseaud (1969). The method is based on the quantification of the conjugation between 1-chlore-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) catalyzed by GST and producing a compound detected at 340 nm (Habig and Jakoby 1981). The GST activity was expressed as μmol CDNB/mg of protein min.

Statistical analyses

Before performing statistical analyses, all results were tested for normality and homogeneity using Kolmogorov-Smirnov and Levene tests, respectively. Levene tests were used based on median values. This method considers the distances of observations from the sample's median instead of the sample's average, which becomes more robust for smaller samples. For all normality tests, the null hypothesis was accepted, and the data were shown to present a normal and uniform distribution. Data were expressed as mean ± standard error. Animals from the Acclimation and Control groups were compared using a *t* test. The abiotic parameters were compared by one-way ANOVA followed by Tukey's post hoc test. The groups exposed to different herbicide concentrations were compared with animals from the Control group using a one-way analysis of variance tests (ANOVA) followed by a post-hoc Dunnett's test. This test compares means from several experimental groups against a control group mean. For all variables of metabolism and oxidative balance (except CAT, which was log-transformed), the ANOVAs were running with raw data. A significance level of 5% ($p < 0.05$) was considered for all analyses. The statistical tests were performed using the Statistical Package for the Social Sciences (SPSS) software for Windows (IBM®, v. 20), PAST statistic software (Hammer et al. 2001) and STATISTICA (2011).

Results

Mortality was not observed in any of the groups studied; therefore, the concentrations used for this study were considered sub-lethal. The pH values, dissolved oxygen, conductivity, and oxidation reduction potential in the animals' maintenance water did not differ ($p < 0.05$) between the experimental groups, during the experiment. Ammonia levels were significantly lower in the aquarium water of animals exposed to a low concentration of sulfentrazone and glyphosate compared with that of the control group (Table 1).

Morphological parameters

In Fig. 1, we can see a boxplot of the length (a) and weight (b) of the animals in the different experimental groups. The body mass of tadpoles did not differ significantly between the initial measurement (when they arrived at the laboratory; day 0) and the measurement after the acclimation, or at the end of the experimental period (control group; day 9). On the other hand, the body length was significantly larger in the acclimation (46%) and control (33%) groups compared with the initial body length. The tadpoles from all exposed groups (day 9) were larger (45–52%) than individuals from day 0 (Table 2).

Intermediate metabolism and oxidative balance parameters

Acclimation and control groups

There was no significant difference in the levels of total proteins and uric acid between the acclimation and control groups. The glycogen levels in the control group increased 2.5 times in comparison with the acclimation group. TBARS levels and CAT activity increased 18 and 5.1 times, respectively, in the control animals compared with the acclimatization group. There was no significant difference in SOD activity between groups; while GST activity decreased (2.9 times) in the control group compared with the acclimation group (Table 3).

Groups exposed to herbicides

A summary of the ANOVA statistical results obtained from *M. admirabilis* tadpoles exposed to the two studied concentrations of Boral® 500 SC (sulfentrazone) and Roundup®

Table 1 Abiotic parameters measured throughout the experimental period in the different groups: acclimation (A)—five days in laboratory, control (C)—nine days in laboratory, sulfentrazone—Boral® 500 SC (B-130 μg a.i./L and B-980 μg a.i./L), and glyphosate—Roundup® Original (R-234 μg a.i./L and R-2340 μg a.i./L). Data are expressed as mean ± standard error. Different letters indicate significant differences ($p < 0.05$) between groups compared with control (C)

Abiotic parameters	A	C	B-130 μg a.i./L	B-980 μg a.i./L	R-234 μg a.i./L	R-2340 μg a.i./L
pH	7.234 ± 0.064[a]	7.155 ± 0.0250[a]	7.062 ± 0.046[a]	7.031 ± 0.050[a]	7.093 ± 0.024[a]	7.093 ± 0.032[a]
Ammonia (ppm)	0.361 ± 0.028[a]	0.437 ± 0.079[a]	0.125 ± 0.043[b]	0.237 ± 0.090[a]	0.138 ± 0.031[b]	0.188 ± 0.055[a]
Dissolved O_2 (mg/L)	4.936 ± 0.108[a]	4.760 ± 0.095[a]	4.978 ± 0.070[a]	4.998 ± 0.092[a]	4.858 ± 0.095[a]	4.920 ± 0.069[a]
Conductivity (μs/cm)	115.876 ± 2.365[a]	125.557 ± 6.006[a]	122.745 ± 4.254[a]	123.625 ± 5.931[a]	117.950 ± 2.355[a]	121.200 ± 5.155[a]
Oxidation reduction potential (mV)	211.766 ± 5.497[a]	217.900 ± 2.542[a]	216.050 ± 11.774[a]	208.200 ± 14.740[a]	212.825 ± 2.469[a]	211.350 ± 4.515[a]

Original (glyphosate) when compared to the control group is presented in Table 4. Detailed comparisons between groups are provided in Table 5. All tested variables related both to metabolism and oxidant balance were influenced by pesticide exposure, except for uric acid levels.

Concerning the intermediate metabolism parameters, the glycogen levels showed a significant difference between the control group, lower glyphosate concentration and both concentrations of sulfentrazone (Fig. 2a and b) (Table 5). Total protein levels increased significantly in tadpoles exposed to B-980 μg a.i./L (420%) and to R-234 μg a.i./L (653%) in comparison with the control group and the other herbicide concentrations (Fig. 2c and d). Uric acid levels did not differ significantly between experimental groups, with similar values between exposed tadpoles and the control group (Fig. 2e and f).

Regarding the oxidative balance parameters, in comparison with the control group, LPO levels decreased in tadpoles

Table 2 Body mass (g) and length (mm) of *Melanophryniscus admirabilis* tadpoles measured at laboratory arrival (initial), after a 5-day acclimation period (acclimation), and at the end of the experimental period (control). Data from animals with no herbicide exposure during the experimental period (control) and exposed to different herbicide concentrations: sulfentrazone—Boral® 500 SC (B-130 μg a.i./L and B-980 μg a.i./L) and glyphosate—Roundup® Original (R-234 μg a.i./L and R-2340 μg a.i./L). $A_{5\ days}$ and $A_{9\ days}$ represent the % of the initial body length by which the given group was longer on average. Results are expressed as mean ± standard error. Different letters indicate significant differences ($p < 0.05$) between groups compared with the initial group. n = number of animals in each group

Groups	n	Body mass (g)	Length (mm)
Initial	90	0.097 ± 0.007[a]	9.971 ± 0.453[b]
Acclimation	15	0.073 ± 0.009[a]	14.555 ± 0.346[a]
			$A_{5\ days}$ = 46.0%
Control	15	0.110 ± 0.038[a]	13.295 ± 0.669[a]
			$A_{9\ days}$ = 33.3%
B-130 μg a.i./L	15	0.086 ± 0.007[a]	14.799 ± 0.388[a]
			$A_{9\ days}$ = 48.4%
B-980 μg a.i./L	15	0.091 ± 0.004[a]	15.158 ± 0.458[a]
			$A_{9\ days}$ = 52.0%
R-234 μg a.i./L	15	0.099 ± 0.010[a]	14.522 ± 0.494[a]
			$A_{9\ days}$ = 45.6%
R-2340 μg a.i./L	15	0.072 ± 0.008[a]	14.531 ± 0.599[a]
			$A_{9\ days}$ = 45.7%

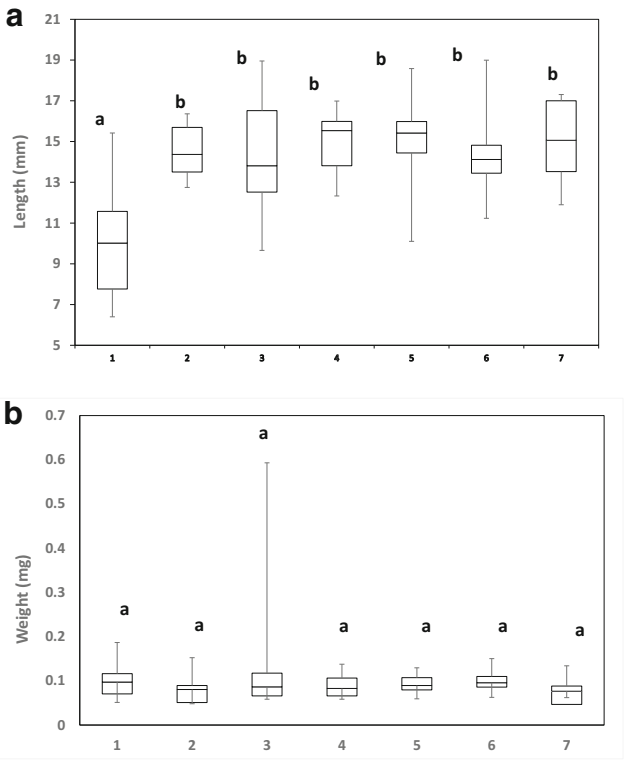

Fig. 1 Effects of Boral® 500SC and Roundup® Original on length (**a**) and weight (**b**) of tadpoles. Different letters indicate significant differences ($p < 0.05$) between the experimental groups. We can classify the animals within each group as follows: 1, initial, animals between stages 25 and 30; 2, acclimation, animals between 31 and 36; 3, exposure control; 4, B-130 μg a.i./L; 5, B-980 μg a.i./L; 6 R-234 μg a.i./; and 7, R-2340 μg a.i./L, animals between 34 and 39

Table 3 Analyzed parameters of *Melanophryniscus admirabilis* tadpoles in the acclimation (A) and control (C) groups. Results are expressed as mean ± standard error. Different letters indicate significant differences ($p < 0.05$) between the groups

Parameters	Groups	
Intermediate metabolism	A	C
Glycogen	318.5 ± 16.6[a]	787.7 ± 19.0[b]
Total protein	71.6 ± 14.5[a]	89.4 ± 29.0[a]
Uric acid	103.4 ± 13.4[a]	148.6 ± 10.0[a]
Oxidative balance		
TBARS	0.2698 ± 0.1515[a]	5.0462 ± 0.6260[b]
SOD	0.2534 ± 0.0087[a]	0.3174 ± 0.0215[a]
CAT	0.1000 ± 0.0303[a]	0.5186 ± 0.0704[b]
GST	9.8307 ± 0.7930[a]	3.3394 ± 0.8520[b]

Glycogen; total protein; uric acid: μg g wet weight^{-1} . Lipid peroxidation (TBARS): μmol mg protein^{-1} ; superoxide dismutase (SOD): U mg protein^{-1} ; catalase (CAT): pmol mg protein^{-1} min^{-1} ; glutathione S-transferase (GST): μmol mg protein^{-1} min^{-1}

Acclimation (A): five days in laboratory

Control (C): nine days in laboratory

exposed to both sulfentrazone concentrations (59% in B-130 μg a.i./L and 95% in B-980 μg a.i./L) (Fig. 3a) and to the highest concentration of glyphosate (61.7% in R-2340 μg a.i./L) (Fig. 3b). SOD levels were significantly lower in both sulfentrazone groups (31% in B-130 μg a.i./L and 33% in B-980 μg a.i./L) and both glyphosate groups (26% in R-234 μg a.i./L and 40% in R-2340 μg a.i./L), when compared with the control group (Fig. 3c and d). CAT activity levels were lower (77%) and varied significantly between tadpoles exposed to lower concentrations of sulfentrazone (B-130 μg/L) in relation to the control group (Fig. 3e, Table 5). We did not observe significant variation in GST activity, despite having increased about 80% in animals exposed to Boral® 550 SC; for animals exposed to the two concentrations of Roundup® Original, we observed a significant increase in this activity (R-234 μg a.i./L: 355% and R-2340 μg a.i./L: 290%) (Fig. 3g and h).

Table 4 Summary of one-way ANOVA tests for metabolic and oxidative balance parameters of *Melanophryniscus admirabilis* tadpoles between the tested groups (control, R1: Roundup® Original (glyphosate) at 234 μg a.i /L; R2: Roundup® Original (glyphosate) at 2340 μg a.i./L; B1: Boral® 500 SC (sulfentrazone) at 130 μg a.i./L; B2: Boral® 500 SC (sulfentrazone) at: 980 μg a.i./L

Parameters	Sum of squares	df1–df2	*F*	*P*
Metabolism				
Glycogen	1.25954	4–20	64.36	< 0.001
Total protein	122.175	4–20	84.76	< 0.001
Uric acid	1.25954	4–20	64.36	< 0.001
Oxidative balance				
TBARS	74.3279	4–29	14.4	< 0.001
SOD	0.0588995	4–25	11.15	< 0.001
CAT	3.35719	4–45	3.142	0.023
GST2	908.519	4–45	26.08	< 0.001

TBARS, lipid peroxidation; SOD, superoxide dismutase; CAT, catalase; GST, glutathione S-transferase; df1, degrees of freedom between groups; df2, degrees of freedom within groups. *F*, value in ANOVA; *p*, α value

Discussion

This is the first study to evaluate aspects of the toxic potential of herbicides on markers of energy metabolism and oxidative balance in tadpoles of *Melanophryniscus admirabilis*, an anuran species with microendemic geographic distribution. Exposure of tadpoles to different concentrations of Boral® 500 SC and Roundup® Original led to glycogen mobilization, increased total protein levels, and maintenance of uric acid levels. We observed a modulation of the antioxidant system that was reflected in the reduction of SOD and CAT levels and the increase of GST activity in animals exposed to both herbicides. This response was accompanied by a decrease in TBARS levels, which was more intense in sulfentrazone-exposed tadpoles than in those exposed to glyphosate.

During our experiments, there was no mortality of tadpoles regardless of the herbicide concentration to which they were exposed, and this finding indicates that the tested herbicide concentrations were sub-lethal to tadpoles during the experiment. The absence of mortality may be related to the use of herbicide concentrations, at least for glyphosate, already found in agricultural areas and/or to the peculiarities of the species studied. Additionally, it is possible to confirm that the experimental conditions throughout the 9-day experiment were suitable for tadpoles and ensured their survival, regardless of the herbicide concentration. All animals, including those in the groups exposed to the herbicides, showed an increase in body length when compared with the initial group. The fact that the animals maintained their ability to grow in terms of body length reinforces the previous statement. As for the weight of the animals, we did not observe a significant increase in any of the groups. This response profile may be associated with the short time it takes to complete the metamorphosis. At the end of the experimental period, about 20% of the animals within each experimental group showed budding or presence of the hind limbs, which may have influenced our results. We observed in the laboratory that *M. admirabilis* takes an average of 15 days from the hatching of the eggs to complete the entire process of metamorphosis, which makes it difficult to work with animals all at the same stage of development.

Table 5 Detailed pairwise-group *P* significance comparisons from one-way ANOVA based on post-hoc Dunnett's test. R1, Roundup® Original (glyphosate) at 234 μg a.i./L; R2, Roundup® Original (glyphosate) at 2340 μg a.i./L; B1, Boral® 500 SC (sulfentrazone) at 130 μg a.i./L; B2, Boral® 500 SC (sulfentrazone) at: 980 μg a.i./L

Metabolism							
Glycogen		Protein		Uric acid			
Groups	*P*	Groups	*P*	Groups	*P*		
B1	**0.000006**	B1	0.056766	B1	0.571822		
B2	**0.000006**	B2	**0.000006**	B2	0.991325		
R1	**0.000006**	R1	**0.000006**	R1	0.735712		
R2	0.181388	R2	0.322543	R2	0.837226		
Oxidative balance							
TBARS		SOD		CAT		GST	
Groups	*P*	Groups	*P*	Groups	*P*	Groups	*P*
B1	**0.000037**	B1	**0.000224**	B1	0.132189	B1	0.278832
B2	**0.000010**	B2	**0.000131**	B2	0.999871	B2	0.145114
R1	0.056832	R1	**0.002353**	R1	0.336938	R1	**0.000007**
R2	**0.000137**	R2	**0.000014**	R2	0.340159	R2	**0.000008**

Significant values are highlighted in bold

TBARS, lipid peroxidation; SOD, superoxide dismutase; CAT, catalase; GST, glutathione S-transferase

The comparison between tadpoles from the acclimation and control groups indicated that protein and uric acid levels were maintained in the acclimation group, while glycogen levels increased two-fold. These results suggest a possible role of glycogen as an energy source to maintain the body while restructuring during the metamorphosis process. The achievement of a minimal energetic status is crucial to ensure that metamorphosis occurs and lasts for a sufficient duration, as demonstrated by Audo et al. (1995) in *Dryophytes chrysoscelis* tadpoles. Anuran metamorphosis occurs along with morphological, physiological, and behavioral alterations, and it is dependent on energy stored during the larval period (Orlofske and Hopkins 2009). Compared with the acclimation group, increased levels of TBARS and CAT activity, coupled with a decrease in GST in the control group, may also be related to the preparation of these animals to cope with the climax of metamorphosis. This is corroborated by the observation of the hind limb budding in 20% of tadpoles and the body mass and length gain at the end of the experiment. Menon and Rozman (2007) suggest that in *Xenopus laevis* tadpoles the cellular environment in the gut and tail becomes progressively more oxidizing during their remodeling and regression, respectively. These authors verified an increase in the levels of TBARS and ascorbic acid, together with a decrease in catalase and glutathione reductase. Further studies that evaluate the relationship between the development of *M. admirabilis* and the oxidative balance parameters are needed.

During the experiments, glycogen levels decreased in a concentration-dependent relationship with sulfentrazone (48% in B-130 μg a.i./L and 71% in B-980 μg a.i./L). Tadpoles exposed to low glyphosate concentration (R-234 μg a.i./L group) had a more pronounced decrease in glycogen content (65%) compared with the control group. This reduction of glycogen levels in tadpoles exposed to herbicides in our experiments suggests an important role of this polysaccharide for ensuring the survival of animals in the presence of xenobiotics. Glycogen is probably used as a substrate for ATP synthesis and/or for the maintenance of circulating glucose levels. Reduction of glycogen levels was also observed in *Ptychadena bibroni* tadpoles exposed to the herbicide atrazine (Ezemonye and Tongo 2009) and in *Rana catesbeiana* exposed to different concentrations of three herbicides (atrazine, glyphosate, and quinclorac) (Dornelles and Oliveira 2014). *Rana temporaria* tadpoles sampled in natural pools near agricultural areas, where glyphosate metabolism products were found, also showed reduced glycogen levels (Strong et al. 2016).

Protein levels were significantly higher in *M. admirabilis* tadpoles exposed to the high sulfentrazone concentration and the low glyphosate concentration when compared with the control group. Bullfrog tadpoles exposed to low glyphosate concentrations (18 and 36 μg/L) have also been shown to exhibit a significant increase in liver and muscle protein levels, as verified by Dornelles and Oliveira (2014, 2016). These authors suggest that the higher protein levels are related to a decrease in protein catabolism and/or an increase in protein synthesis as a response to glyphosate exposure. Based on these results, we can infer that the protein synthesis increase in *M. admirabilis* tadpoles might be an attempt to repair the tissue structure during herbicide exposure. Coltro et al. (2017) also observed an increase in total protein levels in the tail muscle of bullfrog tadpoles exposed to the herbicide quinclorac and suggest that this response may be associated with antioxidant protein synthesis and/or total protein

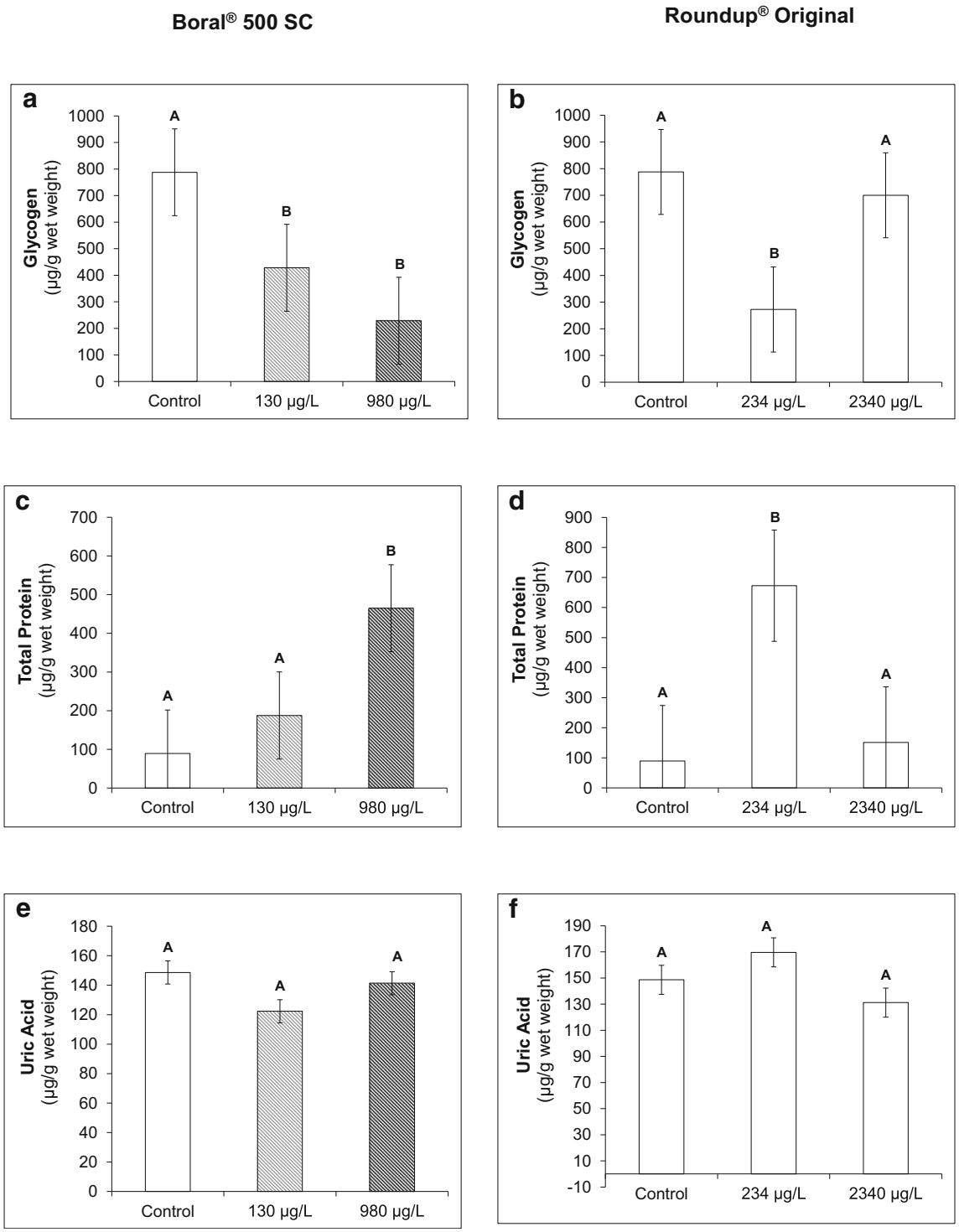

Fig. 2 Total levels of glycogen (**a**, **b**); total proteins (**c**, **d**); and uric acid (**e**, **f**) in *Melanophryniscus admirabilis* tadpoles exposed to different herbicide concentrations: sulfentrazone (Boral® 500 SC: B-130 µg a.i./L and B-980 µg a.i./L) and glyphosate (Roundup® Original: R-234 µg a.i./L and R-2340 µg a.i./L). Results are expressed as mean ± standard error. Different letters indicate significant differences ($p < 0.05$) between the experimental groups

conservation for use in the metamorphosis process. The tail is an important energy source which compensates for the decrease of food intake that occurs during metamorphosis (Albinati et al. 1998). Therefore, the higher levels of total proteins observed in this study may suggest a compensatory mechanism that involves increased protein synthesis to act as an antioxidant (enzymes) and/or as an energy source during the metamorphosis process (tail absorption).

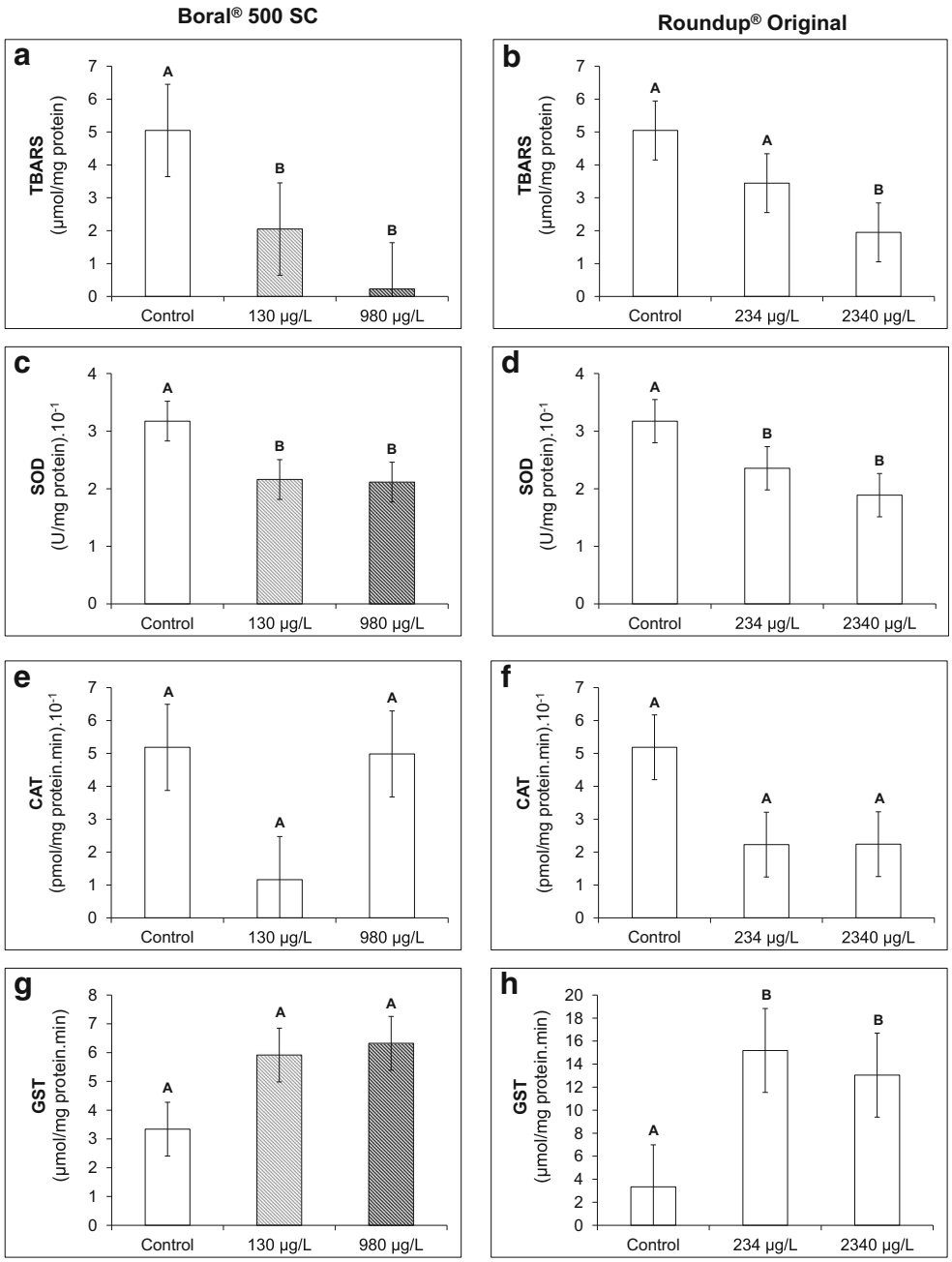

Fig. 3 Levels of lipid peroxidation (TBARS: **a**, **b**) and activity of enzymes superoxide dismutase (SOD: **c**, **d**); catalase (CAT: **e**, **f**); and glutathione S-transferase (GST: **g**, **h**) in *Melanophryniscus admirabilis* tadpoles exposed to different herbicide concentrations: sulfentrazone (Boral® 500 SC: B-130 µg a.i./L and B-980 µg a.i./L) and glyphosate (Roundup® Original: R-234 µg a.i./L and R-2340 µg a.i./L). Results are expressed as mean ± standard error. Different letters indicate significant differences ($p < 0.05$) between the experimental groups

When the tadpoles were exposed to highest glyphosate concentration (R-2340 µg a.i./L), there was a reduction in protein levels relative to the lowest herbicide concentration (R-234 µg a.i./L). This seems to be related to a higher stressor potential of this herbicide when compared with sulfentrazone. Therefore, our results demonstrate that when glycogen levels decreased, proteins were mobilized as a possible compensatory mechanism to cope the energetic demand imposed by the metabolization of the herbicide. As suggested by Umminger (1977), this reduction could also be a result of the activation of protein catabolism complementing glycogen use.

Uric acid levels did not vary significantly between groups regardless of the herbicide concentrations to which the tadpoles were exposed. At the same time, ammonia levels in the aquarium water of animals exposed to B-130 µg a.i./L and R-234 µg a.i./L decreased. Ammonia is normally excreted in the tadpole's gills but can be converted to urea or uric acid in the liver. Uric acid acts as an effective antioxidant and electron donor in xenobiotic detoxification processes as demonstrated by Zanette et al. (2015) for barnacles. This has already been proposed by Coltro et al. (2017) for *Rana catesbeiana* exposed to the herbicide quinclorac, where the authors observed

a decrease in plasma uric acid levels at high concentrations of the herbicide.

Lipid peroxidation levels, expressed as TBARS, were reduced in *M. admirabilis* tadpoles exposed to glyphosate (61.7%) and showed a concentration-dependent pattern of decrease in sulfentrazone groups (51% in B-130 μg a.i./L and 95% in B-980 μg a.i./L). Wilkens et al. (2019) did not find significant changes in TBARS levels in bullfrog tadpoles exposed to Boral (130 μg a.i./L) and glyphosate (234 μg a.i./L). On the other hand, American bullfrog tadpoles exposed to Roundup® Original (1000 μg/L) for 48 h had increased levels of TBARS in liver and muscle, indicating oxidative damage due to ROS excess probably caused by exposure to the herbicide (Costa et al. 2008). Dornelles and Oliveira (2014) studying different bullfrog tissues (gills, liver, and muscle) show that all tissues exhibited increased concentration-dependent lipid peroxidation after seven days of glyphosate exposure (36, 72, and 144 μg/L). Glyphosate and its commercial formulations may increase the chances of LPO occurrence since its compounds can directly interact with plasmatic membranes of cells of different animals (Hazarika et al. 2003; Pérez et al. 2011). However, physiological responses depend on the species and stage of development, the type and concentration of herbicide, and the exposure time (Persch et al. 2017). Therefore, further studies are needed to elucidate if other herbicide concentrations and/or exposure durations would lead to LPO occurrence in *M. admirabilis* tadpoles.

Bufonid poison frogs of the genus *Melanophryniscus* contain alkaloid-based chemical defenses that are derived from diet of alkaloid-containing arthropods. In addition to dietary alkaloids, bufadienolide-like compounds and indolealkylamines have been identified in certain species of *Melanophryniscus* (Hantak et al. 2013; Jeckel et al. 2015). Most of these defensive chemicals are produced endogenously through biosynthesis, but poison frogs sequester lipophilic alkaloids from dietary arthropods. Alkaloid composition varies greatly, even among conspecific individuals collected at the same time and place, with some individuals having only a few micrograms of one or a few alkaloids and others possessing > 1 mg of > 30 alkaloids (Jeckel et al. 2015). Grant et al. (2012) performed an analysis by gas chromatography and mass spectrometry of skin extracts from 11 individuals of *Melanophriniscus simplex* which resulted in the detection of 47 alkaloids (including isomers), 9 unclassified and 38 from 12 known structural classes. Each alkaloid that was present in the skin of an individual was also present in the same relative proportion in that individual's skeletal muscle, liver, and oocytes. The presence of these alkaloids in oocytes can expose embryos to toxic substances early, stimulating the establishment of a more robust antioxidant system and which will be advantageous in adult life when the animal starts to sequester and accumulate these substances from the diet.

These compounds have a toxic potential; therefore, these amphibians had developed the capacity to manipulate and resist the toxic effects of these substances (Santos et al. 2016a). Reinforcing this hypothesis, a reduction in the TBARS levels in our experiments was verified suggesting a high antioxidant capacity of *M. admirabilis*, possibly due to the manipulation capacity of this species, which may have prevented the increase of LPO in tadpoles exposed to the herbicides analyzed in this study. However, further studies are necessary, including the analysis of other oxidative damage biomarkers besides LPO in *M. admirabilis*.

Depending on the intensity, exposure time, affected tissue, and susceptibility of the species exposed to a chemical stressor, the antioxidant activity of enzymes can be intensified or reduced (Oruç and Usta 2007). Our results for *M. admirabilis* corroborate this previous statement, since we verified a different pattern in the antioxidant activity of enzymes depending on the concentration of both tested herbicides.

Superoxide dismutase levels were significantly reduced in *M. admirabilis* tadpoles exposed to different concentrations of both herbicides. Similar results were seen in American bullfrog tadpoles exposed to different concentrations of quinclorac for seven days (Coltro et al. 2017). The fish *Prochilodus lineatus* also demonstrated reduced SOD activity when exposed to Roundup® (10 μg/L) for 24 h (Modesto and Martinez 2010). In contrast, *Bufo bufo gargarizans* tadpoles exposed to different concentrations (30, 60, 130, 650, and 3230 μg/L) of the insecticide Spirotetramat showed a significant increase in SOD activity after four days of exposure, except for at the highest concentration. However, after 15 days, SOD activity in this experiment significantly decreased in the exposed groups (650 μg/L) (Yin et al. 2014). These results might be due to a down-regulation mechanism of the antioxidant system against the exposure to xenobiotics (Sun et al. 2007; Yin et al. 2014).

Catalase activity levels decreased (77%) in *M. admirabilis* tadpoles exposed to the lowest concentration of sulfentrazone (B-130 μg a.i./L); increasing in the highest concentration (B-980 μg a.i./L), but this difference was not significant when compared with the control group. In this study, tadpoles of both tested glyphosate concentrations reduced CAT levels by 57%; however, this reduction was not significant in relation to the control group. This lack of difference in CAT activity has been already observed in *Scinax fuscovarius* tadpoles exposed to different concentrations of the insecticide Fipronil (Margarido et al. 2013). American bullfrog tadpoles exposed to different concentrations of quinclorac had similar responses (Coltro et al. 2017). Different authors show in their studies that not only the levels of lipoperoxidation but also the activity of antioxidant enzymes may increase or decrease depending on the substance to which the animals are exposed. This response also depends on the intensity, duration of exposure, organs analyzed, and the susceptibility of the species studied (Coltro et al. 2017; Oruç

and Usta 2007; Persch et al. 2017, 2018; Wilkens et al. 2019). These results may explain the CAT pattern variation observed in *M. admirabilis* tadpoles. Furthermore, the enzyme glutathione peroxidase (GPx) acts on the metabolism of peroxides, both organic and inorganic (Barata et al. 2005), assuming unique importance in tadpoles and fish exposed to environmental contaminants (Guo et al. 2010; Jones et al. 2010; Modesto and Martinez 2010; Zhu et al. 2015). Therefore, we cannot rule out the possibility that the absence of increased levels of LPO in our experiment is a result of GPx performance, in addition to other adjustments to oxidative balance observed in our experiments. Further studies should clarify the role of GPx in herbicide exposures regarding *M. admirabilis*.

The levels of glutathione S-transferase in *M. admirabilis* tadpoles exposed to the two concentrations of glyphosate increased significantly, reaching values 300% higher than those of the control group. Although GST activity levels also increased in animals from groups exposed to sulfentrazone, these were not significant. Similar results were reported by Santos et al. (2015) in *Phyllomedusa iheringii* tadpoles sampled from natural pools near to soybean and wheat plantations with the probable influence of carbofuran and glyphosate. There was an increase in GST activity in animals sampled from these pools compared with animals sampled from a control area with no herbicidal influence. GST cooperates in the conjugation of endo- and xenobiotic compounds with the glutathione molecule, facilitating the excretion of these substances (Costantini 2014). Hence, this enzyme might play an important role in the homeostasis of *M. admirabilis* tadpoles facing exposure to both herbicides analyzed. GST may have prevented the occurrence of LPO and other possible oxidative damage by decreasing the residence time of herbicides and facilitating their excretion. The absence of mortality observed throughout the experiment reinforces this hypothesis. However, prolonged exposure to herbicides and intense GST activity may deplete the antioxidant system because of the reduction of endogenous glutathione reserves, leading to an oxidative unbalance and compromising the long-term survival of these animals.

Conclusions

The results obtained in our study suggest that *Melanophryniscus admirabilis* has a high antioxidant capacity, which ensured the survival of tadpoles in the tested concentrations of Boral® 500 SC (sulfentrazone: B-130 μg a.i/L and B-980 μg a.i./L) and Roundup® Original (glyphosate: R-234 μg a.i./L and R-2340 μg a.i./L) herbicides. On the other hand, important tested variables related both to oxidative balance and metabolism were statistically influenced by exposure to pesticides. Therefore, although *M. admirabilis* has an effective antioxidant system, exposure of natural populations to pesticides could impose a serious risk to the species, since their restricted distribution, habitat specificity requirements, in addition to the high physiological demand to metabolize xenobiotics. The high physiological cost, in case of chronic or prolonged exposure, can influence several bioecological aspects related to alterations in behavior, fitness, growth, reproductive activity, metamorphosis, and vulnerability to predators. For example, we observed glycogen depletion following exposure to both herbicides indicating this molecule is involved in restoring energy homeostasis during xenobiotic metabolism. The increase of glutathione S-transferase activity, playing a central role in the metabolization process of the two herbicides, could represent a crucial element for the survival of exposed tadpoles. Furthermore, modulation of the antioxidant and the biotransformation system may result in an impaired ability of tadpoles to cope with other stressful environmental conditions. In addition, our results highlighted the need to adopt precautionary measures to avoid contamination by pesticides in the occurrence area of the highly endemic and threatened *M. admirabilis*. In this sense, monitoring and quantification of pesticides in the species habitat, in addition to investigating the origin of these contaminants and their routes, constitutes essential actions to ensure the viability of the population and to subsidize future management and conservation strategies applied to *M. admirabilis*.

Acknowledgments We would like to thank Michelle Abadie, Thayná Mendes, Juliane Heyde, and Matheus Kingeski for their sample support and information on the species studied, and Luis Esteban Krause Lanes (PUCRS) for his valuable help in the statistical analysis of the work. We thank the Fundação Grupo O Boticário and RAN/ICMBio for their financial and logistical support and the National Council for Scientific and Technological Development (CNPq) for granting a master's degree grant to the first author and for providing a productivity grant to the corresponding author.

Authors contributions Conceptualization: Guendalina Turcato Oliveira, Márcio Borges-Martins, and Patrícia Rodrigues da Silva. Data curation: Guendalina Turcato Oliveira, Márcio Borges-Martins, and Patrícia Rodrigues da Silva. Formal analysis: Guendalina Turcato Oliveira, Márcio Borges-Martins, and Patrícia Rodrigues da Silva. Funding acquisition: Guendalina Turcato Oliveira and Márcio Borges-Martins. Investigation: Guendalina Turcato Oliveira and Patrícia Rodrigues da Silva. Methodology: Guendalina Turcato Oliveira, Márcio Borges-Martins, and Patrícia Rodrigues da Silva. Project administration: Guendalina Turcato Oliveira. Resources: Guendalina Turcato Oliveira and Márcio Borges-Martins. Software: Guendalina Turcato Oliveira. Supervision: Guendalina Turcato Oliveira and Márcio Borges-Martins. Validation: Guendalina Turcato Oliveira and Márcio Borges-Martins. Visualization: Guendalina Turcato Oliveira, Márcio Borges-Martins, and Patrícia Rodrigues da Silva. Writing—original draft: Guendalina Turcato Oliveira, Márcio Borges-Martins, and Patrícia Rodrigues da Silva. Writing—review and editing: Guendalina Turcato Oliveira, Márcio Borges-Martins, and Patrícia Rodrigues da Silva.

Funding This study was funded by the Fundação Grupo O Boticário and RAN/ICMBio, the National Council for Scientific and Technological Development (CNPq) (case number 307071/2015-4), and the Higher Education Personnel Improvement Coordination—Brazil (CAPES) (Financial Code 001).

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

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

Ethical approval All procedures involving animals were authorized by the Committee on Animal Research and Ethics from the Pontifícia Universidade Católica do Rio Grande do Sul (CEUA/PUCRS) (Permit n° 6879). Sampling size and procedures were authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBio) (Permit n° 40004-4) and followed the legal precepts of the Federative Republic of Brazil.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

Adamu KM, Kori-Siakpere O (2011) Effects of sub lethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust on some biochemical parameters of hybrid catfish (*Clarias gariepinus* and *Heterobranchus bidorsalis*). Braz Arch Biol Technol 54:183–196. https://doi.org/10.1590/S1516-89132011000100023

Albinati RCB, Costa GB, Neves AP (1998) Efeito da densidade populacional de girinos de (*Rana catesbeiana* Shaw, 1802) sobre o tempo de metamorfose e peso do ímagos. Arq Esc Med Vet UFBA 19:75–86

Albuquerque AF, Ribeiro JS, Kummrow F, Nogueira AJA, Montagner CC, Umbuzeiro GA (2016) Pesticides in Brazilian freshwaters: a critical review. Environ Sci Process Impacts 18:779–787. https://doi.org/10.1039/c6em00268d

AmphibiaWeb (2020) Amphibian species lists. University of California, Berkeley http://amphibiaweb.org. Accessed 03 July 2020

Annett R, Habibi HR, Hontela A (2014) Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. J Appl Toxicol 34:458–479. https://doi.org/10.1002/jat.2997

ANVISA—Agência Nacional de Vigilância Sanitaria (2005) Nota técnica sobre livre comércio de agrotóxicos e impactos à saúde humana. http://www.anvisa.gov.br. Accessed 03 July 2020

Audo MC, Mann TM, Polk TL, Loudenslager CM, Diehl WJ, Altig R (1995) Food deprivation during different periods of tadpole (*Hyla chrysoscelis*) ontogeny affects metamorphic performance differently. Oecologia 103:518–522. https://doi.org/10.1007/BF00328691

Avigliano L, Fassiano AV, Medesani DA, De Molina MR, Rodríguez EM (2014) Effects of glyphosate on growth rate, metabolic rate and energy reserves of early juvenile crayfish, *Cherax quadricarinatus*. M Bull Environ Contam Toxicol 92:631–635. https://doi.org/10.1007/s00128-014-1240-7

Barata C, Lekumberri M, Vila-Escalé M, Prat N, Porte C (2005) Trace metal concentration, antioxidant enzyme activities and susceptibility to oxidative stress in the tricoptera larvae *Hydropsyche exocellata* from the Llobregat river basin (NE Spain). Aquat Toxicol 74:3–19. https://doi.org/10.1016/j.aquatox.2005.04.002

Beckie HJ, Flower KC, Ashworth MB (2020) Farming without glyphosate? Plants 9. https://doi.org/10.3390/plants9010096

Bókony V, Mikó Z, Mórick ÁM, Kruzselvi D, Hettyey A (2017) Chronic exposure to a glyphosate-based herbicide makes toad larvae more toxic. Proc R Soc B 284:20170493. https://doi.org/10.1098/rspb.2017.0493

Botta F, Lavison G, Couturier G, Alliot F, Moreau-Guigon E, Fauchon N, Guery B, Chevreuil M, Blanchoud H (2009) Transfer of glyphosate and its degradate AMPA to surface waters through urban sewerage systems. Chemosphere 77:133–139. https://doi.org/10.1016/j.chemosphere.2009.05.008

Boveris A, Cadenas E (1982) Production of superoxide radicals and hydrogen peroxide in mitochondria. In: Oberley LW (ed) Superoxide dismutase. CRC Press, Florida, pp 15–30

Boveris A, Chance B (1973) The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochem J 134:707–716. https://doi.org/10.1042/bj1340707

Boyland E, Chasseaud LF (1969) The role of glutathione and glutathione S-transferases in mercapturic acid biosynthesis. Adv Enzymol Relat Areas Mol Biol 32:172–129. https://doi.org/10.1002/9780470122778.ch5

Buege JA, Aust SD (1978) Microsomal lipids peroxidation. Meth Enzymol 52:302–310. https://doi.org/10.1016/S0076-6879(78)52032-6

Collins JP, Storfer A (2003) Global amphibian declines: sorting the hypotheses. Divers Distrib 9:89–98. https://doi.org/10.1046/j.1472-4642.2003.00012.x

Couture G, Legris J, Langevin R (1995) Evaluation des impacts du glyphosate utilisedans le milieu forestier. Ministere des Ressources Naturelles, Direction de Penviron- ment forestier, Service du suivi environnemental, Charles- bourg, Quebec, Canada.

Coltro ML, Silva PR, Valgas ANN, Miguel C, Freitas BS, Oliveira GT (2017) Influence of the herbicide Facet® on corticosterone levels, plasma metabolites, and antioxidant system in the liver and muscle of American bullfrog tadpoles. Water Air Soil Pollut 228:241. https://doi.org/10.1007/s11270-017-3404-7

CONCEA—Conselho Nacional de Controle de Experimentação Animal (2013) Diretrizes da Prática de Eutanásia do CONCEA. Ministério da Ciência, Tecnologia e Inovação, Brasília

Costa MJ, Monteiro DA, Oliveira-Neto AL, Rantin FT, Kalinin AL (2008) Oxidative stress biomarkers and heart function in bullfrog tadpoles exposed to roundup original. Ecotoxicology 17:153–163. https://doi.org/10.1007/s10646-007-0178-5

Costantini D (2014) Oxidative stress and hormesis in evolutionary ecology and physiology. Springer, New York

Dowling DK, Simmons LW (2009) Reactive oxygen species as universal constraints in life-history evolution. Proceedings of the Royal Society B: Biological Sciences 276 (1663):1737–1745

Di-Bernardo M, Maneyro R, Grillo H (2006) New species of *Melanophryniscus* (Anura: Bufonidae) from Rio Grande do Sul, Southern Brazil. J Herpetol 40:261–266. https://doi.org/10.1670/05-008.1

Dornelles MF, Oliveira GT (2014) Effect of atrazine, glyphosate, and quinclorac on biochemical parameters, lipid peroxidation and survival in bullfrog tadpoles (*Lithobates catesbeianus*). Arch Environ Contam Toxicol 66:415–429. https://doi.org/10.1007/s00244-013-9967-4

Dornelles MF, Oliveira GT (2016) Toxicity of atrazine, glyphosate, and quinclorac in bullfrog tadpoles exposed to concentrations below legal limits. Environ Sci Pollut Res 23:610–1620. https://doi.org/10.1007/s11356-015-5388-4

Dutra de Armas E, Rosim Monteiro RT, Valler Amâncio A, Lopes Correa RM, Guercio MA (2005) Uso de agrotóxicos em cana-de-açúcar na bacia do rio Corumbataí e o risco de poluição hídrica. Quim Nova 28:975–982. https://doi.org/10.1590/S0100-40422005000600008

Edwards W M, Triplett G B, Kramer RM (1980) A watershed study of glyphosate transport in runoff. J. Environ. Qual., 9, 661-665

Emater-RS. 2001. Relatório de ações e resultados 2001. Escritório municipal de Arvorezinha – RS.

Endemann M, Hristoforoglu K, Stauber TE, Wilhelm E (2001) Assessment of age-related polyploidy in *Quercus robur* L. somatic embryos and regenerated plants using DNA flow cytometry. Biol Plant 44:339–345. https://doi.org/10.1023/A:1012426306493

Ezemonye L, Tongo I (2009) Lethal and sublethal effects of atrazine to amphibian larvae. Jordan J Biol Sci 2:29–36

Fonte LFM, Abadie M, Mendes T, Zank C, Borges-Martins M (2014) The times they are a-changing: how a multi-institutional effort stopped the construction of a hydroelectric power plant that threatened a critically endangered red-belly toad in southern Brazil. FrogLog 22:18–21

Freitas JS, Teresa FB, de Almeida EA (2017) Influence of temperature on the antioxidant responses and lipid peroxidation of two species of tadpoles (*Rhinella schneideri* and *Physalaemus nattereri*) exposed to the herbicide sulfentrazone (Boral 500SC®). Comp Biochem Phys C Toxicol Pharmacol 197:32–44

Galbraith DW, Harkins KR, Maddox JM, Ayres NM, Sharma DP, Firoozabady E (1983) Rapid flow cytometric analysis of the cell-cycle in intact plant-tissues. Science 220:1049–1051

Gehrke VR, Camargo ER, Avilla LA (2020) Sulfentrazone: Environmental Dynamics and Selectivity. Planta Daninha 38

Gill JPK, Sethi N, Mohan A, Datta S, Girdhar M (2018) Glyphosate toxicity for animals. Environ Chem Lett 16:401–426. https://doi.org/10.1007/s10311-017-0689-0

Gosner KLA (1960) Simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190

Govindarajulu PP (2008) Literature review of impacts of glyphosate herbicide on amphibians: what risks can the silvicultural use of this herbicide pose for amphibians in BC? B.C. Ministry of Environment, Victoria, BC Wildlife Report No. R-28

Grant T, Colombo P, Verrastro L, Saporito RA (2012) The occurrence of defensive alkaloids in non-integumentary tissues of the Brazilian red-belly toad *Melanophryniscus simplex* (Bufonidae). Chemoecology 22(3):169–178. https://doi.org/10.1007/s00049-012-0107-9

Gripp HS, Freitas JS, Almeida EA, Bisinoti MC, Moreira AB (2017) Biochemical effects of fipronil and its metabolites on lipid peroxidation and enzymatic antioxidant defense in tadpoles (*Eupemphix nattereri*: Leiuperidae). Ecotoxicol Environ Saf 136:173–179. https://doi.org/10.1016/j.ecoenv.2016.10.027

Grube A, Donaldson D, Kiely T, Wu L (2011) Pesticides industry sales and usage. US EPA, Washington, DC

Guo H, Yin L, Zhang S, Feng W (2010) The toxic mechanism of high lethality of herbicide butachlor in marine flatfish flounder, *Paralichthys olivaceus*. J Ocean Univ China 9:257–264. https://doi.org/10.1007/s11802-010-1693-1

Habig WH, Jakoby WB (1981) Glutathione S-transferases (rat and human). Meth Enzymol 77:218–234. https://doi.org/10.1016/S0076-6879(81)77029-0

Halliwell B, Gutteridge JMC (2007) Free radicals in biology and medicine. Oxford University Press, New York

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4:1–9

Hantak MM, Grant T, Reinsch S, Loring M, Toyooka N, Saporito RA (2013) Dietary alkaloid sequestration in a poison frog: an experimental test of alkaloid uptake in *Melanophryniscus stelzneri* (Bufonidae). J Chem Ecol 39:1400–1406. https://doi.org/10.1007/s10886-013-0361-5

Hazarika A, Sarkar SN, Hajare S, Kataria M, Malik JK (2003) Influence of malathion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. Toxicology 185:1–8. https://doi.org/10.1016/S0300-483X(02)00574-7

Hermes-Lima M (2004) Oxygen in biology and biochemistry: role of free radicals. In: Storey KB (ed) Functional metabolism: regulation and adaptation. Hoboken, New York, pp 319–368

ICMBio—Instituto Chico Mendes de Conservação da Biodiversidade (2012) Sumário Executivo do Plano de Ação Nacional para Conservação de Répteis e Anfíbios Ameaçados da Região Sul do Brasil. Centro Nacional de Pesquisa e Conservação de Répteis e Anfíbios (RAN), Brasília http://www.icmbio.gov.br/portal/images/stories/docs-plano-de-acao/pan-herpetofauna-sul/sumarioherpetofaunasul-web.pdf. Accessed 03 July 2020

ICMBio—Instituto Chico Mendes de Conservação da Biodiversidade (2018). Livro Vermelho da Fauna Brasileira Ameaçada de Extinção: Volume V—Anfíbios. In: Instituto Chico Mendes de Conservação da Biodiversidade (Org.). Livro Vermelho da Fauna Brasileira Ameaçada de Extinção. Brasília: ICMBio

IUCN (2013) The International Union for Conservation of Nature. Amphibians on the IUCN red list of threatened species. http://www.iucnredlist.org. Accessed 03 July 2020

Jeckel MA, Grant T, Saporito RA (2015) Sequestered and synthesized chemical defenses in the poison frog *Melanophryniscus moreirae*. J Chem Ecol 41:505–512. https://doi.org/10.1007/s10886-015-0578-6

Jones L, Gossett DR, Banks SW, McCallum ML (2010) Antioxidant defense system in tadpoles of the American bullfrog (*Lithobates catesbeianus*) exposed to paraquat. J Herpetol 44:222–228. https://doi.org/10.1670/07-243.1

Legris J, Couture G (1989) Residus de glyphosate dans l'eau et les sediments suite a des pulverisations terrestres en milieu forestier en 1986. Publication #3322. Gouvernement du Quebec, Ministere de l'Energie et des Ressources, Direction de la Conservation, Charlesbourg, Quebec, Canada

Lima ES, Abdalla DSP (2001) Peroxidação lipídica: mecanismos e avaliação em amostras biológicas. Braz J Pharm Sci 37:293–303

Llesuy SF, Milei J, Molina H, Boveris A, Milei S (1985) Comparison of lipid peroxidation and myocardia damage induced by adriamycin and 4′-epiadrimicin in mice. Tumor 71:241–249

Marcelo Hermes-Lima, Kenneth B. Storey, (1993) In vitro oxidative inactivation of glutathione S-transferase from a freeze tolerant reptile. Molecular and Cellular Biochemistry 124 (2):149-158

Margarido TCS, Felicio AA, Rossa-Feres DC, Almeida EA (2013) Biochemical biomarkers in *Scinax fuscovarius* tadpoles exposed to a commercial formulation of the pesticide fipronil. Mar Environ Res 91:61–67. https://doi.org/10.1016/j.marenvres.2013.02.001

Melo CAD, Medeiros WN, Tuffi Santos LD, Ferreira FA, Ferreira GL, Paes FASV, Reis MR (2010) Residual effect of sulfentrazone, isoxaflutole and oxyfluorfen in three soils. Planta Daninha 28:835–842. https://doi.org/10.1590/S0100-83582010000400017

Menon J, Rozman R (2007) Oxidative stress, tissue remodeling and regression during amphibian metamorphosis. Comp Biochem Physiol C Toxicol Pharmacol 145:625–631. https://doi.org/10.1016/j.cbpc.2007.02.011

Miller JI (1997) Pesticide fact sheet, registration of a new chemical. US Environmental Protection Agency, Washington, DC

Modesto KA, Martinez CBR (2010) Roundup® causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. Chemosphere 78:294–299. https://doi.org/10.1016/j.chemosphere.2009.10.047

Monaghan P, Neil B, Metcalfe NB, Torres R (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecology Letters 12:75–92. https://doi.org/10.1111/j.1461-0248.2008.01258.x

Montgomery MP, Kamel F, Saldana TM, Alavanja MCR, Sandler DP (2008) Incident diabetes and pesticide exposure among licensed pesticide applicators: agricultural health study 1993–2003. Am J Epidemiol 167:1235–1246. https://doi.org/10.1093/aje/kwn028

Moutinho MF, de Almeida ED, Espíndola ELG, Daam MA, Schiesari L (2020) Herbicides employed in sugarcane plantations have lethal and sublethal effects to larval *Boana pardalis* (Amphibia, Hylidae). Ecotoxicology. https://doi.org/10.1007/s10646-020-02226-z

Newton M, Horner LM, Cowell JE, White DE, Cole EC (1994) Dissipation of Glyphosate and Aminomethylphosphonic Acid in North American Forests. Journal of Agricultural and Food Chemistry 42 (8):1795-1802

Nunes C, Silva A, Soares E, Ganias K (2011) The use of hepatic e somatic indices e histological information to characterize the reproductive dynamics of Atlantic sardine Sardina pilchardus from the Portuguese coast. Mar. Coast. Fish. 3: 127-144

Ojha A, Yaduvanshi SK, Srivastava N (2011) Effect of combined exposure of commonly used organophosphate pesticides on lipid peroxidation and antioxidant enzymes in rat tissues. Pestic Biochem Physiol 99:148–156. https://doi.org/10.1016/j.pestbp.2010.11.011

Orlofske SA, Hopkins WA (2009) Energetics of metamorphic climax in the pickerel frog (*Lithobates palustris*). Comp Biochem Physiol A Mol Integr Physiol 154:191–196. https://doi.org/10.1016/j.cbpa.2009.06.001

Oruç EÖ, Usta D (2007) Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. Environ Toxicol Pharmacol 23:48–55. https://doi.org/10.1016/j.etap.2006.06.005

Peres F, Moreira JC (2003) É veneno ou é remédio? Agrotóxicos, saúde e ambiente. SciELO—Editora Fiocruz, Rio de Janeiro

Pérez GL, Vera MS, Miranda LA (2011) Effects of herbicide glyphosate and glyphosate-based formulations on aquatic ecosystems. In: Kortekamp A (ed) Herbicides and environment. https://www.intechopen.com/books/herbicides-and-environment/effects-of-herbicide-glyphosate-and-glyphosate-based-formulations-on-aquatic-ecosystems. Accessed 18 July 2020

Persch TSP, Weimer RN, Freitas BS, Oliveira GT (2017) Metabolic parameters and oxidative balance in juvenile *Rhamdia quelen* exposed to rice paddy herbicides: Roundup®, Primoleo®, and Facet®. Chemosphere 174:98–109. https://doi.org/10.1016/j.chemosphere.2017.01.092

Persch TSP, Silva PR, dos Santos SHD, de Freitas BS, Oliveira GT (2018) Changes in intermediate metabolism and oxidative balance parameters in sexually matured three-barbeled catfishes exposed to herbicides from rice crops (Roundup®, Primoleo® and Facet®). Environ Toxicol Pharmacol 58:170–179. https://doi.org/10.1016/j.etap.2018.01.004

Peruzzo PJ, Porta AA, Ronco AE (2008) Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in North Pampasic region of Argentina. Environ Pollut 156:61–66. https://doi.org/10.1016/j.envpol.2008.01.015

Relyea RA (2005) The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecol Appl 15: 618–627. https://doi.org/10.1890/03-5342

Rodrigues BN, de Almeida FS (1998) Guia de herbicidas. Iapar, São Paulo

Santos EA, Correia MN, Silva JRM, Velini ED, Passos ABRJ, Durigan JC (2015) Herbicide detection in groundwater in Córrego Rico-SP watershed. Planta Daninha 33(1):147–155. https://doi.org/10.1590/S0100-83582015000100017

Santos JC, Tarvin RD, O'Connell LA (2016a) A review of chemical defense in poison frogs (Dendrobatidae): ecology, pharmacokinetics, and autoresistance. In: Buesching CD (ed) Chemical signals in vertebrates. Springer, New York

Santos LFJ, Oliveira-Bahia VRL, Nakaghi LSO, De Stefani MV, Gonçalves AM, Pizauro Junior JM (2016b) Ontogeny of the digestive enzymes of tadpoles of *Lithobates catesbeianus*. Copeia 104(4): 838–842. https://doi.org/10.1643/CG-16-432

Schier M, Chandel N (2014) Function in redox signaling and oxidative stress. Curr Biol 24(10):R453–R462. https://doi.org/10.1016/j.cub.2014.03.034

Schiesari L, Grillitsch B, Grillitsch H (2007) Biogeographic biases in research and their consequences for linking amphibian declines to pollution. Conserv Biol 21:465–471. https://doi.org/10.1111/j.1523-1739.2006.00616.x

Segalla MV, Caramaschi U, Cruz CAG, Grant T, Haddad CFB, Garcia PCA, Berneck BVM, Langone JA (2016) Brazilian amphibians—list of species. Herpetol Bras 3:37–48

Sies H (2018) On the history of oxidative stress: Concept and some aspects of current development. Current Opinion in Toxicology 7: 122–126

SINDAG—Sindicato Nacional das Indústrias de Defensivos Agrícolas (2011) Dados de produção e consumo de agrotóxicos. http://www.sindag.com.br. Accessed 03 July 2020

STATISTICA Enterprise- Statsoft South America. Software Statistica. Statsoft, v.6.0, 2011

Strong RJ, Halsall CJ, Ferenčík M, Jones KC, Shore RF, Martin FL (2016) Biospectroscopy reveals the effect of varying water quality on tadpole tissues of the common frog (*Rana temporaria*). Environ Pollut 213:322–337. https://doi.org/10.1016/j.envpol.2016.02.025

Sun Y, Yin G, Zhang J, Yu H, Wang X (2007) Bioaccumulation and ROS generation in liver of freshwater fish, goldfish *Carassius auratus* under HC Orange no. 1 exposure. Environ Toxicol 22:256–263. https://doi.org/10.1002/tox.20262

Taysse L, Chambras C, Marionnet D, Bosgiraud C, Deschaux P (1998) Basal level and induction of cytochrome P450, EROD, UDPGT, and GST activities in carp (*Cyprinus carpio*) immune organs (spleen and head kidney). Bull Environ Contam Toxicol 60:300–305. https://doi.org/10.1007/s001289900625

Thorngren JL, Harwood AD, Murphy TM, Hartz KEH, Fung CY, Lydy MJ (2017) Fate and risk of atrazine and sulfentrazone to nontarget species at an agriculture site. Environ Toxicol and Chem 36(5): 1301–1310. https://doi.org/10.1002/etc.3664

Tsahar E, Arad Z, Izhaki I, Guglielmo CG (2006) The relationship between uric acid and its oxidative product allantoin: a potential indicator for the evaluation of oxidative stress in birds. J Comp Physiol B Biochem Syst Environ Physiol 176:653–661. https://doi.org/10.1007/s00360-006-0088-5

Umminger BL (1977) Relation of whole blood sugar concentration in vertebrate to standard metabolic rate. Comp Biochem Physiol—Part A Physiol 56:457–460. https://doi.org/10.1016/0300-9629(77)90267-5

Van Handel E (1965) Estimation of glycogen in small amount soft tissue. Anal Biochem 11:256–265

van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology 13 (2):57-149

Vasconcellos M A de (2015) Conservação do sapinho-admirável-de-barriga-vermelha, Melanophryniscus admirabilis (anura: bufonidae) : estudo de ecologia populacional. Dissertation, Universidade Federal do Rio Grande do Sul

Wagner N, Reichenbercher W, Teichmann Z, Tappeser B, Lottersy S (2013) Questions concerning the potential impact of glyphosate-based herbicides on amphibians. Environ Toxicol Chem 32(8): 1688–1700. https://doi.org/10.1002/etc.2268

Walker CH (2014) Ecotoxicology: effects of pollutants on the natural environment. CRC Press, New York

Wilkens ALL, Valgas AAN, Oliveira GT (2019) Effects of ecologically relevant concentrations of Boral® 500 SC, Glifosato® Biocarb, and a blend of both herbicides on markers of metabolism, stress, and nutritional condition factors in bullfrog tadpoles. Environ Sci Poll Res 26:23242–23256. https://doi.org/10.1007/s11356-019-05533-z

Yin X, Jiang S, Yu J, Zhu G, Wu H, Mao C (2014) Effects of spirotetramat on the acute toxicity, oxidative stress, and lipid peroxidation in Chinese toad (*Bufo bufo gargarizans*) tadpoles. Environ

Toxicol Pharmacol 37:1229–1235. https://doi.org/10.1016/j.etap.2014.04.016

Zanette J, Monserrat JM, Bianchini A (2015) Biochemical biomarkers in barnacles *Balanus improvisus*: pollution and seasonal effects. Mar Environ Res 103:74–79. https://doi.org/10.1016/j.marenvres.2014.11.001

Zhang W, Chen L, Xu Y, Deng Y, Zhang L, Qin Y, Wang Z, Liu R, Zhou Z, Diao J (2019) Amphibian (*Rana nigromaculata*) exposed to cyproconazole: changes in growth index, behavioral endpoints, antioxidant biomarkers, thyroid and gonad development. Aquat Toxicol 208:62–70. https://doi.org/10.1016/j.aquatox.2018.12.015

Zhu L, Mu X, Wang K, Chai T, Yang Y, Qiu L, Wang C (2015) Cyhalofop-butyl has the potential to induce developmental toxicity, oxidative stress and apoptosis in early life stage of zebrafish (*Danio rerio*). Environ Pollut 203:40–49. https://doi.org/10.1016/j.envpol.2015.03.044