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Roundup Original DI® and thermal stress affect survival, morphology and thermal tolerance in tadpoles of *Boana faber* (Hylidae, Anura)

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Abstract

In amphibians, stressful environments can lead to accelerated metamorphosis at the expense of total length, resulting in the occurrence of morphological abnormalities. Many studies have linked the occurrence of these phenomena to the pollution of habitats by pesticides and thermal stress. Here, we assessed how exposure to Roundup Original DI® and higher constant temperatures affect the survival of *Boana faber* tadpoles and estimate the CL5096hs for the population. In addition, we evaluated how exposure to Roundup affects larval growth, morphology and thermal tolerance. Our findings suggest that even at sublethal doses, Roundup Original DI® may affect the survival of *Boana faber* larvae. There also appears to be an additive effect between Roundup and temperature increase on larval survival, however, we need to further explore this point to determine a pattern, proving to be a promising issue to be investigated in the future. We observed effects of chronic exposure to the herbicide formulation on the morphology and growth of the tadpoles, resulting in a reduction in total length and differences in the shape of the larvae. Although we did not recover any direct effects of herbicide exposure on CTMax, we did observe an upward trend in CTMax for tadpoles exposed to Roundup. Understanding how anthropogenic changes affect anuran persistence is fundamental for the management and conservation of the species and can be considered an initial step toward the formulation of legislations that regulate the use of herbicides.

Keywords Ecotoxicology · Amphibians · Development · Temperature · Roundup · Herbicide

Introduction

From a historical perspective, many researchers consider the current biodiversity crisis as a sixth mass extinction event (Wake and Vredenburg 2008). Human-induced climate change and other anthropogenic stressors, such as environmental degradation and habitat contamination, have been

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identified as potential causes of species' decline, affecting their persistence and increasing the likelihood of extinction (Stuart et al. 2004; Wake and Vredenburg 2008). However, understanding biodiversity-specific responses to multiple stressors has been a complex challenge (Katzenberger et al. 2014). Hence, to accurately predict how species can cope with a changing environment, we need to understand how human-induced stressors (e.g., pesticide contamination) can affect species' response to other abiotic stressors (e.g., increasing temperatures; Pörtner and Farrell 2008).

Many species have the ability to respond to changing environmental conditions with environmentally induced phenotypes (Ghalambor et al. 2007), in which the differential expression of a single genotype can potentially generate several phenotypic variations (Pigliucci 2001). This phenotypic plasticity allows individuals to "track" and adjust their allocation of resources, promoting changes in their traits (Atkinson and Thompson 1987; Denver 2021). Hence, phenotypic plasticity can manifest itself adaptively if the induced-phenotype increases species persistence, and on longer timescales it can promote evolution to a new phenotype favored by directional selection (Ghalambor et al. 2007). However, some stressors may induce sub-optimal phenotypes or interact non-synergistically with other stressors, hampering the expression of the optimal phenotype for certain environmental conditions and potentially resulting in non-adaptive plasticity (Katzenberger et al. 2014).

Growth and development rates usually show high phenotypic plasticity in response to changing environments (Rohr et al. 2011). In general, amphibian larvae exposed to stressful environments tend to have shorter development times and faster growth rates, resulting in a reduced size at metamorphosis when compared to conspecifics raised in optimal environments (Alvarez and Nicieza 2002; Benard 2004; Gomez-Mestre et al., 2010, Kulkarni et al. 2011; Newman 1987). Although this strategy favors the species' persistence under stressful environments, it can also entail physiological and morphological costs (Gomez-Mestre et al. 2013). The acceleration of ontogenetic development can cause morphological abnormalities in tadpoles (e.g., Bolis et al. 2020; Bridges 1999; Costa and Nomura 2016; Egea-Serrano et al. 2012), potentially reflecting on fitness components such as growth, longevity and survival (Møller 1997).

As species are usually exposed to multiple stressors in their natural environment, the interaction between stressors may promote changes different from those induced by each stressor alone, as seen between temperature and chemical pollution (Freitas and Almeida 2016; Freitas et al. 2017a, b; Katzenberger et al. 2014). The climate-mediated toxic sensitivity hypothesis predicts that increased metabolism caused by higher temperatures can increase the impact of pollutants on ectotherms, increasing energy expenditure to maintain the body's functionalities (Hooper et al. 2013). Increased metabolic rates, as an effort to detoxify pollutants such as heavy metals or agrochemicals from their organism, eventually limit the ability of tadpoles to respond to increasing water temperatures (Hallman and Brooks 2015). Moreover, physiological performance of ectotherms is well known to be linked to environmental temperatures and may be limited when oxygen availability is low (Pörtner 2001). When standard maintenance costs are low, the biological relevance of this effect may be relatively low. However, if maintenance costs of the metabolic rates (i.e., oxygen demand) increase due to higher temperatures while being exposed to contaminants, its energy demand may become too high and the water-dissolved oxygen may not be sufficiently available to meet the respiratory requirements. Increased energy demand can therefore indirectly constrain the upper thermal tolerance in these organisms (Hallman and Brooks 2015).

A wide variety of chemical contaminants such as herbicides, insecticides and pesticides have been introduced into natural environments through direct application, urban sewage and artificial runoff (Egea-Serrano et al. 2012). Glyphosate-based herbicides have been used worldwide to control agricultural and non-agricultural weeds. The increase in the use of this herbicide has been reflected in the high detection of glyphosate and its AMPA degradation in rivers and streams located in the United States (Medalie et al. 2020; USGS 2022), Argentina (Ronco et al. 2008) and Brazil (Lima et al. 2022); the three countries with the highest consumption of glyphosate-based herbicides in the world (Richmond 2018).

Moreover, the latest IPCC report (2022) predicts the occurrence of unprecedented extreme events, increases in mean temperature and changes in precipitation patterns over the next few decades. Hence, considering the increasing use of chemical contaminants and the current scenario of climate change, it is important to assess how species' ability to cope with environmental temperatures will be affected by the presence of pesticides. In this context, we estimated the CL50-(96 h) and evaluated how exposure to the herbicide Roundup Original DI® affects survival, growth and thermal tolerance in *Boana faber (Wied-Neuwied, 1821)* tadpoles. In addition, we assessed how the combined exposure to sublethal doses of Roundup Original DI® and higher constant temperatures affects larvae survival.

Considering that at higher temperatures tadpoles are usually under increased metabolic demands (Angilletta 2009; McDiarmid and Altig, 1999), we expect an increased mortality of tadpoles exposed to higher concentrations of Roundup Original DI® and higher constant temperatures, as allocation of available energy would have to cope with both stressors simultaneously. We also expect tadpoles exposed to sublethal doses of Roundup Original DI® to present herbicide-induced morphological changes, as seen in other amphibian species (Costa and Nomura 2016; Katzenberger et al. 2014;

Relyea 2012). In addition, as suggested by the climate-mediated toxic sensitivity hypothesis (Hallman and Brooks 2015), we hypothesize that tadpoles exposed to Roundup Original DI® should have lower critical thermal maximum values, indicating a lower tolerance to temperature.

Material and methods

Clutch collection and experimental system

The blacksmith tree frog (*Boana faber* (Wied-Neuwied, 1821)) is widely distributed in South America, and can be found in forest, open canopy or anthropic environments (Martins and Haddad 1988). We collected a total of eight egg masses of *B. faber*, on two separate nights, from a lentic water body (14°55'19,8"S e 39°01'30,4"W, Fig. 1) located at the indigenous territory "Tupinambá de Olivença" in the municipality of Ilhéus, Bahia, Brazil. Clutches collected in March 13, 2021, were used to assess Roundup Original DI®'s LC50-(96 h) and its combined effect with temperature on tadpole survival, whereas those collected in November 11, 2020, were used to determine Roundup Original DI®'s effect on tadpole morphology and critical thermal maximum (CTmax). On each occasion, we transported the egg masses to the Tropical Herpetology Laboratory at the Universidade Estadual de Santa Cruz (UESC). Egg masses were then pooled together in a single storage tank (60 cm × 40 cm × 40 cm) containing 6 liters of dechlorinated water. Once hatched, tadpoles were fed daily ad libitum with Alcon colors ® ornamental fish feed. Experiments were carried out in the laboratory, under controlled conditions (25 °C, 12 h light/12 h dark, except when mentioned otherwise) using glass aquariums (60 cm × 40 cm × 40 cm) as experimental units with 2 L of dechlorinated water and without substrate or vegetation. The larvae were kept in the laboratory until they reached stage 25 of development (Gosner 1960).

We used a commercial formulation (Roundup Original DI®) with 48% of active ingredient as a contaminant in all experiments. The half-life of glyphosate in the environment can reach up to 21 days (Giesy et al. 2000). Thus, experiments had a single application of the contaminant within the period evaluated. Concentrations used in acute and chronic exposures were based on previous studies regarding the effect of Roundup Original DI® on anuran larvae (Bolis et al. 2020; Costa and Nomura 2016; Daam et al. 2019; Herek et al. 2020; Lima et al. 2020; Moutinho et al. 2020). The concentrations can be considered environmentally realistic, considering that concentrations higher than those tested in

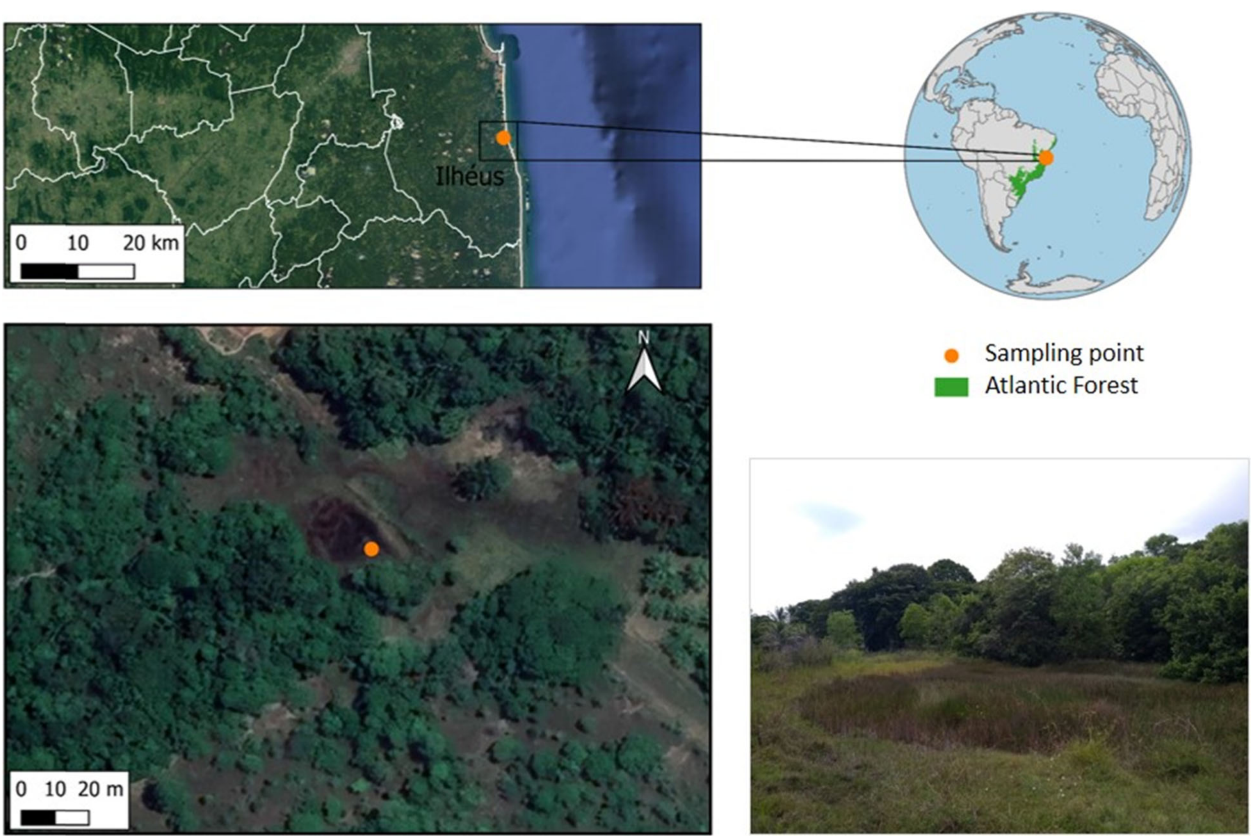

Fig. 1 Location of the water body where the spawning of a population of *Boana faber* was collected

our experiment were found in rivers and streams located in Brazil (Lima et al. 2022) and Argentina (Ronco et al. 2008).

Experiment 1: tadpole survival and estimation of LC50-(96 h) of Roundup Original DI®

After 20 days of development (Gosner stage 25, Gosner 1960), tadpoles were divided into groups, each of which exposed to a different Roundup Original DI® concentration: 0 mg a.i./L (control), 2, 4, 6 and 8 mg a.i./L. In the control treatment, we applied a dose of 25 uL of water to avoid any potential manipulating bias. We added 10 tadpoles to each aquarium and replicated each treatment six times, totaling 30 experimental units and 300 tadpoles. Aquariums were surveyed every 24 h for 4 consecutive days (96 h), to count the number of surviving tadpoles and remove the deceased ones. At the end of the experiment (96 h), surviving tadpoles were counted and euthanized using an aqueous solution of xylocaine and fixed in 10% formalin. All individuals were added to the scientific collection of the Universidade Estadual de Santa Cruz (MZUESC). Treatment replicates were randomly assigned to each aquarium and pH was measured both at the beginning and the end of the experiment.

Experiment 2: effect of temperature and sublethal exposure to Roundup Original DI® on tadpole survival

To evaluate the effect of temperature and a sublethal dose of Roundup Original DI®, we used a 2 × 2 design with temperature (25° and 32 °C) and Roundup Original DI® (nominal concentrations of 0 and 5.58 mg a.i/L) as factors. The sublethal concentration used represented 75% of LC50-(96 h) and was based on preliminary observations and later confirmed by the previous experiment. We used *Hot* aquarium heaters (5 W) in combination with *Ageon* thermostats to keep the water temperature constant. After 30 days of development (Gosner stage 25, Gosner 1960), 15 tadpoles were assigned to each aquarium and each treatment was replicated five times, totaling 20 experimental units and 300 tadpoles. Treatment replicas were randomly assigned to each aquarium. Every 24 h, dead individuals were removed from the experimental units. After 14 days of experiment, surviving tadpoles were counted and euthanized.

Experiment 3: effects of sublethal exposure to Roundup Original DI on tadpole morphology and critical thermal maximum

To assess the effects of Roundup Original DI® on tadpole morphology and critical thermal maximum, we assembled 10 aquariums, five of which had a nominal dose of Roundup Original DI® of 5.58 mg a.i/L and five had 0 mg a.i/L (two treatments). After 30 days of development (stage 25, Gosner 1960), 20 tadpoles were randomly allocated to each aquarium (i.e., 200 tadpoles tested). Larvae were kept at room temperature (25 °C) and were fed ad libitum daily. Every 24 h, dead individuals were removed from the experimental units. After 10 days of experiment, surviving tadpoles were counted and allocated to each experimental procedure, as follows.

Tadpole morphology

After finishing the experiment, we randomly selected 5 tadpoles from each experimental unit to perform morphological analyses, totaling 25 tadpoles per treatment. These tadpoles were placed in a small aquarium with ultrasound gel and water to obtain images in lateral views. For image capture, we used a Leica microscope coupled with a digital camera. We analyzed the images using the ImageJ software, measuring the tadpole's total length (TTL), body length (BL), tail length (TL), maximum tail height (TMH), and tail musculature height (TMH) (Supplementary Table S1). Shape-variation in tadpoles was also measured with geometric morphometric techniques, using a Procrustes superimposition method. Lateral images of 25 tadpoles per treatment were compiled using the tpsUtil software (Rohlf 2009). To assess differences in shape, we digitized 22 landmarks and semilandmarks (see Supplementary Fig. S2) using the tpsDig2 software (Rohlf 2008). To define the landmarks, we used a modified version of Katzenberger et al. (2014) due to morphological differences between *B. faber* and the reference species (*Dryophytes versicolor*).

Maximum critical temperature

To obtain the CTMax and assess whether exposure to Roundup Original DI® affects the individual's thermal tolerance, we followed the protocol proposed by Lutterschmidt and Hutchison (1997). We exposed 12 tadpoles from each treatment in experiment 3 to a constant heating rate of 0.25 °C/min^{-1} until they reached the critical thermal maximum. The end point of the experiment was defined by the lack of reaction of the tadpoles (total immobility) to external stimuli (Gutiérrez-Pesquera et al. 2016). After testing, tadpoles were transferred to water at room temperature (approximately 25 °C), to allow for recovery. Individuals that died within 24 h were excluded from the analysis as we considered that they could overestimate the CTMax.

Statistical analysis

In experiment 1, we used a probit regression (Finney 1971) to estimate the lethal concentration of Roundup Original DI® for 50% of the population- LC50-(96 h). To conduct the analysis, we used the LC_probit function from the *ecotox* package

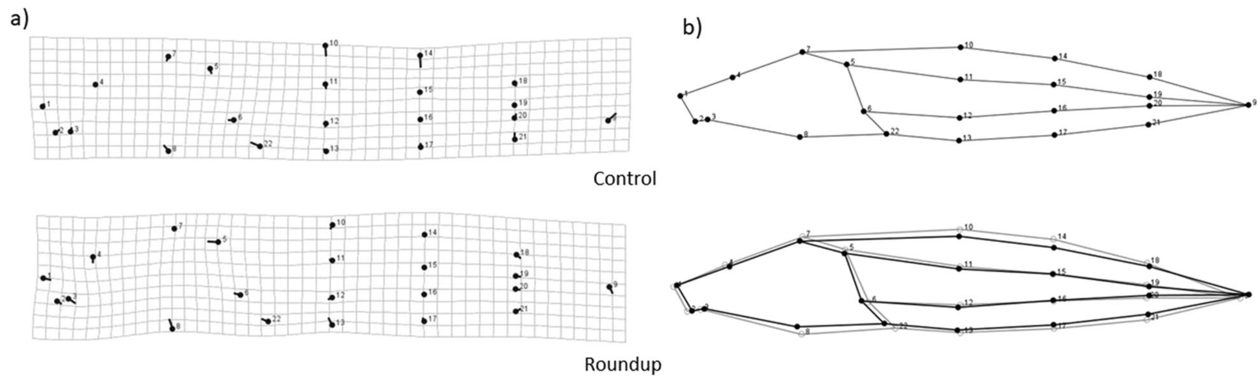

Fig. 2 **a** Transformation grids with landmarks (black dots) and vectors showing the direction of variation; **b** comparison of shape designs for each treatment. The lines represent the shape of the Procrustes consensus of tadpoles exposed to control (0 mg a.i./L) and Roundup Original DI® (5.58 mg a.i./L)

(Hlina et al. 2021). We also performed an ANOVA one-way to assess whether survival (percentage of survivors) differed between treatments. We evaluated the effect of temperature and Roundup Original DI® on larval survival (square root of percentage of survivors, experiment 2) using a two-way ANOVA, which includes each stressor as a categorical predictor and the interaction between both stressors.

In experiment 3, the herbicide effect on larvae survival was evaluated using a one-way ANOVA. We performed a Pearson Correlation test to assess the existence of correlation between linear measurements. As all linear measurements were correlated with the TTL of the tadpoles (Supplementary Table S2), we used TTL as a proxy for size. We conducted a one-way ANOVA to assess the effect of Roundup on TTL. We also performed a discriminant analysis, using MorphoJ software (Klingenberg 2011), to assess whether exposure to Roundup Original DI® induced tadpole shape variation. We conducted a generalized linear model (GLM) to assess the effect of Roundup Original DI® on the thermal tolerance of the tadpoles, using mass as covariate. All analyses were conducted in the program R (R Development Core Team 2022) and at a significance level of alpha = 0.05, except when mentioned otherwise.

Permission

All experiments were approved by the Ethics Committee on Animal Use of the Universidade Estadual de Santa Cruz (process n° 018/19) and followed the guidelines issued by the Brazilian National Council for the Control of Animal Experiments (CONCEA). The animals were collected and transported under authorization from SISBIO-ICMBio (n° 73441-1).

Results

In experiment 1, we observed differences in tadpole survival between treatments ($F_{(4)} = 5.24$, $p = 0.003$; Table 1).

Table 1 Tadpole survival (%) between treatments testing different concentrations of Roundup Original DI®

Treatment	Survival (%)
Control (0 mg a.i/L)	98.33 ± 4.08
T1 (2 mg a.i/L)	93.33 ± 12.11
T2 (4 mg a.i/L)	98.32 ± 4.07
T3 (6 mg a.i/L)	55 ± 45.93
T4 (8 mg a.i/L)	43.33 ± 41.31

The estimated LC50-(96 h) for the population of *Boana faber* was 7.45 mg a.i./L (15.5 uL/L). During this experiment, pH and water temperature were kept constant (pH = 6.8 ± 0.58; temperature = 25.69 ± 0.65 °C). In experiment 2, both Roundup Original DI® ($F_{(1)} = 12.38$, $p < 0.01$, Table 2) and higher water temperature ($F_{(1)} = 48.38$, $p < 0.001$) decreased tadpole survival. Moreover, the interaction between Roundup Original DI® and higher water temperature caused higher tadpole mortality ($F_{(1)} = 7.72$, $p < 0.01$) than in the other treatments.

Similarly to the previous experiments, exposure to Roundup Original DI® in experiment 3 caused a reduction in their tadpole survival ($F_{(1)} = 16.33$, p value = 0.004, Table 3). In addition, we observed an effect of Roundup Original DI® in tadpole size ($F_{(1)} = 34.69$, $p < 0.001$, Table 3), with those being exposed to Roundup Original DI® (15.57 ± 1.78 mm TTL) being smaller than those in the control group (18.56 ± 1.81 mm). Moreover, exposure to Roundup affected larval morphology (procrustes distance = 0.0267, $t = 1221.84$, $p = 0.004$, Fig. 2) inducing shallower bodies for larvae exposed to the contaminant when compared to the control. Finally, neither Roundup Original DI® (estimate = 0.503, $t = 2.065$, $p = 0.057$; Table 3) or mass (estimate = 0.881, $t = 0.234$, $p = 0.818$) affected tadpole's critical thermal maximum (CTMax).

Table 2 Impact of exposure to Roundup Original DI® and temperature on the survival of *Boana faber* larvae

Treatment	Survival (%)
Control (25 °C + 0 mg a.i/L)	42.66 ± 25.21
T1 (32 °C + 0 mg a.i/L)	12 ± 12.82
T2 (25 °C + 5.58 mg a.i/L)	34.66 ± 22.80
T3 (32 °C + 5.58 mg a.i/L)	0 ± 0

Table 3 Effect of sublethal doses of Roundup Original DI® on the survival, total length and thermal tolerance of *Boana faber* larvae

Variable	Control	Treatment
CTMax (°C)	41.54 ± 0.35	42.03 ± 0.59
Total length (mm)	18.56 ± 1.81	15.57 ± 1.78
Survival (%)	100 ± 0	79 ± 10.24

Discussion

Estimating LC50-(96 h) is an important step in understanding how susceptible a species can be to a particular contaminant. Our 7.45 mg a.i./L estimate for LC50-(96 h) (at 25 °C) suggests that *Boana faber* may be more tolerant to Roundup Original DI® than several other amphibians, for example when compared to the LC50-(96 h) of nine species of anurans (values ranging from 0.8 to 2.3 mg a.e./L) and four salamanders (values ranging from 2.7 to 3.2 mg a.e./L) exposed to Roundup Original Max® (48.7% glyphosate) (Relyea and Jones, 2009; Jones et al. 2010). However, due to methodological differences between studies (e.g., mesocosms or laboratory experiments, different densities), comparisons of LC50 estimates among species are not so straightforward, making it difficult to classify which species are most susceptible to the herbicide (Mann et al. 2009).

Both aforementioned studies used Roundup Original Max®, whereas in this study we used Roundup Original DI®. It has been suggested that different formulations of glyphosate-surfactant may vary in toxicity to organisms (Mann et al. 2009; Relyea and Jones 2009), including that the surfactant present in commercial formulations of Glyphosate can cause more damage than the active ingredient itself and increase the toxicity of the herbicide (Giesy et al. 2000). The different glyphosate formulations have different surfactants that can cause different responses in larval anurans. For example, Mann et al. (2009) tested the effect of two different glyphosate formulations on tadpoles of *Crinia insignifera*, *Heleioporus eyrei*, *Limnodynastes dorsalis*, and *Litoria moorei* and found that the original Roundup formulation is more toxic to the larval stage of these species than the glyphosate formulations Touchdown and Roundup Biactive. The toxicity of glyphosate formulations can also vary between species and between different larval stages (Howe et al. 2004). Herek et al. (2020) found a LC50-(96 h) (test temperature 23 °C, Roundup Original DI®) of 1.13 mg a.e./L for *Physalaemus gracilis* (Leptodactylidae) and of 1.06 mg a.e./L for *Physalaemus cuvieri* (Leptodactylidae), whereas Costa and Nomura (2016) also estimated the LC50-(96 h) (test temperature 28 °C, Roundup Original with 48% active ingredient) of *Physalaemus cuvieri* larvae as 2.13 mg a.i./L, but it is unclear whether the difference between the two estimates for *Physalaemus cuvieri* derives solely from population variation in herbicide tolerance. Some species, such as *Xenopus laevis* (Pipidae) embryos (24.75 mg a.e./L at 23 °C, Roundup Power 2.0®) (Bonfanti et al. 2018) or *Rhinella arenarum* (Bufonidae) tadpoles (78.18 mg/L at 20 °C, Credit®) (Carvalho et al. 2019), present high LC50-(96 h), but again the formulations of glyphosate-based herbicides used were different. Our data also shows a strong additive effect between the higher constant temperature and exposure to Roundup® on the survival of *Boana faber* larvae. This constitutes another methodological issue that is pervasive when comparing LC50 estimates, considering the variation in the test temperature used across studies. Since the negative effect of Roundup Original DI® on *B. faber* survival is exacerbated at higher temperatures, it is plausible that LC50 estimates for other amphibian species can also vary depending on which temperature they are conducted at.

In the absence of Roundup Original DI®, survival also decreased in the treatment with higher temperature, despite some mortality in the lower temperature treatment. While both temperatures are within the range of environmental temperatures experienced by *B. faber*, 25 °C is a more commonly experienced temperature, whereas 32 °C is at the higher end of the thermal spectrum for this species (see Gutiérrez-Pesquera et al. 2016; Katzenberger 2014; Simon et al. 2015). However, these temperatures were maintained constant for almost 2 weeks, which hardly occurs in nature. Tadpoles exposed to higher temperatures may experience greater oxidative stress as a consequence of accelerated metabolism and increased demand for oxygen (Freitas and Almeida 2016; Margarido et al. 2013), particularly if there is no daily temperature fluctuation that can provide a temporary reprieve from those higher temperatures. Hence, despite having an estimated optimum temperature for swimming of 32.12 °C (Katzenberger 2014) and critical thermal maximum between 40.5 and 42.3 °C (Simon et al. 2015, Gutiérrez-Pesquera et al. 2016, this study), exposure to constant temperature (particularly at 32 °C) may have affected tadpole survival. Considering the current global warming scenario, with expected increases in mean air temperatures (IPCC 2022) and in the frequency and magnitude of extreme temperature events such as heat waves (Schär et al. 2004), it is important to further assess how temperature affects susceptibility of glyphosate and its impact on amphibian survival.

An additional concern that pertains to climate change, is whether chemical contaminants can affect the thermal physiology of organisms, in particular ectotherms. In our experiments, we did not find an effect of Roundup Original DI® either, but there was a trend toward an increase in CTMax for individuals of *Boana faber*. One possibility would be that exposure to Roundup Original DI® induces the production of heat shock proteins (Hsp) or other metabolic changes (Chen et al. 2018; Melo et al. 2020; Wang et al. 2019) that could indirectly result in the increase in CTMax. Katzenberger et al. (2014) demonstrated that exposure to Roundup Power Max®, a different Roundup® formulation, does not affect the CTMax of *Dryophytes versicolor* larvae but can affect the shape of its thermal performance curve. Although both species belong to the same amphibian family (Hylidae), they are from different genus, which may also explain differences we found in the effect of Roundup® on the CTMax of tadpoles. Moreover, Patra et al. (2007) demonstrated that exposure to the insecticide Endosulfan, an organochlorine insecticide and acaricide, can reduce CTMax in fish. Hence, current knowledge suggests that the effect of chemical contaminants on the thermal physiology of ectotherms may depend on the type and concentration of the contaminant used, on its formula and the mode of action on organisms (Katzenberger et al. 2014).

Exposure to Roundup Original DI® not only affected larvae survival, but induced phenotype changes on *Boana faber* tadpoles, resulting in a reduction in tadpole total length and shallower bodies when exposed to the herbicide. Studies show that herbicide-induced changes can happen in the same direction as phenotypes induced by exposure to predators (Relyea 2012), as shallower bodies and deeper tails can direct the predator's attention to less lethal regions reducing the risk of be killed (Relyea 2003, 2004, 2005; Relyea 2012; Van Buskirk 2002). Stressors such as predators, salinity and habitat modification can affect in different ways the thermal physiology of species, even oppositely, either by constraining or increasing CTMax (Cheng et al. 2022; Chuang et al. 2022; Katzenberger et al. 2014). A possible hypothesis for this similarity in the morphological change induced by different stressors is that the herbicide can induce the production of stress hormones that would also be activated by the presence of predators (Relyea 2012). However, although previous studies have evaluated the effect of herbicides on the shape of tadpoles, the mechanisms that mediate change have not yet been determined (Katzenberger et al. 2014). Although phenotypic plasticity in metamorphic features can be considered a way to cope with stressful environmental conditions, it can result in morphological, physiological and life history costs for the larvae (Gomez-Mestre et al. 2013). Smaller size in metamorphosis can impact sexual selection and the reproductive success of adults, affecting mating success and the number and size of eggs produced by females (Hayes et al. 2010; Mays et al. 2006). Therefore, even if individuals manage to survive under sublethal concentrations of herbicide, their fitness may eventually be reduced, reflecting in the long-term persistence of the local population.

Conclusion

Our findings suggest that Roundup Original DI® reduces survival of *Boana faber* tadpoles, an effect that is exacerbated at higher temperatures. However, since current estimates are being obtained at a panoply of test temperatures, there is a need to standardize LC50 estimation procedures. This would ease comparisons among taxa and provide a better perspective on which species are more vulnerable to Roundup Original DI®. The morphological changes seen in induced-phenotype tadpoles are in line with previous studies (Costa and Nomura 2016; Katzenberger et al. 2014; Relyea 2012), but it is unclear whether different formulations of glyphosate-based herbicides can affect CTMax. Considering the current climate change scenario, it is fundamental to further current understanding of the interactions of Roundup Original DI® with temperature and its effect on the thermal physiology of amphibians. Several stressors can act in synergism with thermal conditions and the presence of pollutants, such as the presence of predators (Katzenberger et al. 2014) and exposure to salinity (Chuang et al. 2022). This would greatly benefit the conservation of amphibian populations, particularly when managing conservation units and the surrounding areas, and contribute additional information for the decision-making process when producing legislation that regulates the use of herbicides and mitigates the influence of other stressors.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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