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In situ works on ecotoxicology in amphibians: current state of the art

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Abstract. Amphibians play a fundamental role in the functioning of ecosystems, mainly in the nutrient cycle, energy flow, and pest control. This group has a massive population decline, with approximately 41% of all species at risk, highlighting pesticide pollution as one of the main threats to the loss of anuran biodiversity. This occurs since amphibians have an epidermis with high permeability and a biphasic life cycle, where they use both the aquatic and terrestrial environments, allowing exposure and making them susceptible to chemical agents released into the environment. This makes amphibians a model group in ecotoxicological studies. However, due to the greater control of experimental conditions, the vast majority of studies are carried out in the laboratory, thus maintaining a gap in knowledge about the effects of pesticides on amphibians in natural environments. Under natural conditions, where environmental variables are not controlled, experiments must relate all the dynamics of the interaction of substances existing in the environment, and their influences on organisms. To evaluate the studies already performed under natural conditions, a bibliographic survey was carried out through a search with the Web of Science and SCOPUS databases, considering articles published in the last twenty years, using the terms 'field experimentation' or '*in situ* study', associated with 'tadpole' and 'agrochemical' or 'pesticide', using a descriptive methodology. Twenty-one abstracts were pre-selected, they were further evaluated and the methodologies were checked to ensure only the analysis of experiments conducted in the field conditions. Thus, ten works were selected for the construction of the review. The works selected used native amphibian species, considered generalists, present in the study region, and were carried out in environments intended for agricultural cultivation with control groups in preserved locations. In these works, different methodologies were used, such as intentional spraying of water bodies, quantification of pesticides in the study sites, and verification of changes caused by the environment on tadpoles. The main evaluations were based on the survival and morphological changes of the tadpoles and, in some cases, on cytological and biochemical assays.

Keywords: Anura, tadpoles, pesticides, field condition.

Introduction

Brazilian law, through Resolution 001 of the National Council for the Environment (CONAMA) of 1986, defines environmental impact as “any change in the physical, chemical and biological properties of the environment caused by any form of matter or energy resulting from human activities that, directly or indirectly affect the health, safety, and well-being of the population, social and economic activities, the biota, the aesthetic and sanitary conditions of the environment and the quality of environmental resources”. The presence of different types of chemicals in water bodies mainly occurs as a result of industrialization (Rojas-Hucks et al., 2019) and agriculture (Guida et al., 2018; Araujo et al., 2019). However, currently, one of the main environmental impacts comes from the use of pesticides used in agricultural environments. Pesticides are widely described as causing negative environmental effects, such as population declines of non-target species such as birds and aquatic organisms (Spadotto et al., 2004; Mahmood et al., 2016), being considered a direct threat to biodiversity loss. of amphibians (Haddad et al., 2013; Sánchez-Bayo & Wyckhuys, 2019; IUCN, 2022).

In rural landscapes, pesticides have raised awareness as they pose a high risk to amphibian communities (Ortiz-Santaliestra et al., 2018). One of the reasons for the great susceptibility of the amphibian group to pesticides released into the environment is due to their biphasic life cycle, which requires aquatic and terrestrial habitats for reproduction and feeding (Haddad et al., 2013; Glinski et al., 2018; Gonçalves et al., 2019). By using the aquatic environment, amphibians have a high permeability of the epidermis (Nyström et al., 2007; Llewelyn et al., 2019), which makes them more exposed to contaminants present in water resources.

Several studies have shown morphological, biochemical, and behavioral changes (Venturino et al., 2003; Nwani et al., 2010; Rutkoski et al., 2020; 2021), mutagenic effects on erythrocyte cells (Gonçalves et al., 2015; Pavan et al., 2021), changes in embryonic development, delaying or inhibiting its metamorphosis (Lanctôt et al., 2014; Bonfanti et al., 2018), reduced reproductive success (Herek et al., 2020), among others, in organisms susceptible to pesticides, which can compromise the amphibian population. In a broad approach, due to the wide variety of responses to environmental stressors, many species of the Anura group are considered bioindicators and are often used as models for ecotoxicological studies (Gonçalves et al., 2019). In addition, frogs are easy to find and capture, have a high degree of fidelity to the place of reproduction (philopatry), and are relatively easy to assess malformations and mortality caused by pesticide contamination (Bank et al., 2005; 2006).

Toxicity tests are important methods for detecting and evaluating the harmful effects of pesticides on non-target species (Silva et al., 2013). In general, most ecotoxicological tests are carried out under controlled conditions, with the analysis of isolated compounds such as pesticides. As the vast majority of studies take place in the laboratory, there is a lack of knowledge regarding the effects that agrochemicals cause on anurans in natural environments (*in situ*). This limits knowledge about the actual effects of chemicals on the environment, as organisms are exposed to complex mixtures of pesticides that may have effects exacerbated by other environmental characteristics (Matson et al., 2009). Thus, it is important to expand the experiments performed in the natural environments of non-target organisms, considering all the dynamics of the interaction of substances existing in the environment, the environmental variables, and the influences they cause on the organisms (Agostini et al., 2020). Despite *in situ* experimentation, using tadpoles as a model organism has been used to outline an ecotoxicological panorama in contaminated environments. However, there are still few studies carried out with amphibians after exposure to pesticides when compared to other taxonomic groups (Ilha & Schiesari, 2014; Ortiz-Santaliestra et al., 2018).

Unlike the controlled conditions of a laboratory, where most studies evaluate isolated pesticides, *in situ* experiments use lakes exposed to pesticides and ponds not exposed or with minimal exposure, the latter being considered reference lakes (Agostini et al., 2020). To evaluate the effects of pesticides on anurofauna *in situ*, several methods are adopted. One of these methods was performed by Agostini et al. (2020), who studied 91 points, in three different areas, of which 71 were adjacent to agricultural environments and 20 ponds without nearby agricultural plots. This allowed having an environment as a reference (preserved location) in order to make it possible to compare the samples. To compare these two sites, 5 enclosures were placed per environment with 20 tadpoles (Gosner stage 29 to 42) for a period of time of 7 days. After these 7 days, there was a scheduled application of pesticides, and after this application, samples were collected in 24 hours (first evaluation) and 48 hours (second evaluation). The authors found that survival was reduced in ponds with pesticide detection.

Another method is the enclosure of tadpoles *in situ*. Agostini et al. (2020) found that the use of this method, with cloistered tadpoles obtained from the same ponds where the closure took place, proved to be effective for *in situ* work, as it facilitates the work methodology. It demonstrates the importance of expanding the study methodologies, in order to consider the real ecological relevance of the real situation of natural environments. *In situ* experiments, using anuran tadpoles as a model, have been used to outline an ecotoxicological panorama in contaminated environments. This methodology considers all the dynamics of the interaction of substances existing in the environment, such as complex mixtures of pesticides, environmental variables, and the influences they cause on the body (Matson et al., 2009; Agostini et al., 2020).

Thus, the aim of this review was to investigate, through a broad methodological review, research studies that evaluated the impact of pesticides on the biology of anurans in the larval stage, based on *in situ* experimentation studies in the last twenty years.

Materials and Methods

The work consisted of bibliographic research carried out using the Web of Science and SCOPUS databases. Initially, articles published from studies performed in the last 20 (twenty) years on the themes 'field experiment', 'tadpole', and 'pesticide' were located, for which a descriptive methodology was used. From all the articles found, there was a pre-selection of articles considering the 'response of tadpoles exposed to pesticides in the natural environment, through field experiments, using as a reference the expressions 'field experimentation' or '*in situ* study', associated with 'tadpole' and 'agrochemical' or 'pesticide'. The inclusion criteria for studies were: (a) performed in the field; (b) studies carried out in the period from 2000 to 2021; (c) publication in a scientific journal; (d) articles in Portuguese, Spanish and English.

Results and Discussion

The present study aimed to review works carried out in the field, focusing on the responses of *in situ* amphibians exposed to pesticides. Considering the keywords used, 1,398 publications were found in the Web of Science and SCOPUS databases between 2000 and 2021. After applying exclusion criteria, 21 articles were read in detail, mainly considering the methodological issues used and, of these, 10 articles were selected for the construction of this manuscript, 20% from Web of Science and 80% from SCOPUS (80%), which are summarized in Table 1.

Table 1. Summary of the main works presented in this research, summarizing the species studied, the location of the study, which pesticides were found, and the effects identified by the respective researchers.

Species	Environment	Pesticides in the environment	Response on tadpoles	References
Boana pulchellus, Leptodactylus latrans, Rhinella arenarum, Rhinella fernandezae	Soybean	Cypermethrin + glyphosate, cypermethrin + glyphosate + endosulfan, chlorpyrifos + glyphosate endosulfan, glyphosate + 2,4-D, glyphosate	Survival, mobility	Agostini et al. (2020)
Leptodactylus mystacinus, Scinax squalirostris	Rice	Lambda-cyhalothrin	Survival, development; cholinesterase and glutathione *S*-transferase activities, cell morphology	Attademo et al. (2014)
Rhinela arenarum	Fruit orchards	Pesticide drift, azinphos-methyl	Carboxylesterase, glutathione and glutathione *S*-transferase activities	Rosenbaum et al. (2012)
Litoria aurea	Industrial use	Not specified	Survival	Pollard et al. (2016)
Rana [*Lithobates*] *pipiens*	Maize; soybean; sorghum	Atrazine; total neonicotinoids; glyphosate	Survival, development, hatching	Dyck et al. (2021)
Rana [*Lithobates*] *clamitans, Rana sylvatica* [*Lithobates sylvaticus*]	-	Glyphosate	Survival, development	Edge et al. (2014)
Rana [*Lithobates*] *clamitans, Rana* [*Lithobates*] *pipiens*	Pesticide spraying	Glyphosate	Survival	Thompson et al. (2004)
Rana [*Lithobates*] *clamitans, Rana* [*Lithobates*] *pipiens*	Pesticide spraying	Triclopyr-2-butoxyethyl ester	Survival, development, mobility	Wojtaszek et al. (2005)
Pseudacris regilla	Agricultural Fields	Chlordanes, endosulfan, chlorpyrifos, malathion, trifluralin, chlorothalonil, hexachlorocyclohexane	Survival, malformations, development, cholinesterase activity, cytometry	Sparling et al. (2014)
Bufo bufo	Agricultural Fields	Not specified	Survival, gonadal differentiation, and development	Orton & Routledge (2011)

The environments used for the *in situ* experiments involved environments intended for general agricultural cultivation (Orton & Routledge 2011; Sparling et al., 2014), fruit orchards (Rosenbaum et al., 2012), rice cultivation (Attademo et al., 2014), soybean (Agostini et al., 2020; Dyck et al., 2021), maize and sorghum (Dyck et al., 2021), in addition to industrial use areas (Pollard et al., 2016) and water bodies with controlled spraying of pesticides (Thompson et al., 2004; Wojtaszek et al., 2005; Edge et al., 2014).

Among the main methodologies used to assess the effects of pesticides present in the environment, the survival and growth of tadpoles stand out (Thompson et al., 2004; Wojtaszek et al., 2005; Orton & Routledge, 2011; Edge et al. 2014; Attademo et al., 2014; Sparling et al., 2014; Pollard et al., 2016; Agostini et al. 2020; Dyck et al., 2021). In addition to these analyses, hatch success (Dyck et al., 2021), changes in mobility (Wojtaszek et al. 2005; Agostini et al. 2020), malformations (Thompson et al 2004), cytometry (Thompson et al., 2004), cell morphology (Attademo et al. 2014) and biochemical markers such as carboxylase (Rosenbaum et al., 2012), cholinesterase (Attademo et al., 2014; Thompson et al., 2004), glutathione (Rosenbaum et al., 2012), and glutathione-*S*-transferase (Rosenbaum et al., 2012; Attademo et al., 2014) activities.

All studies discussed the presence of pesticides in the environments under study. Four of these, despite not having quantified the levels of pesticides present in the environment, considered the history of the area and the possible presence of pesticides resulting from application in agricultural fields or rice fields (Orton & Routledge 2011, Attademo et al., 2014), in fruit orchards (Rosenbaum, 2012), and industrial use environments (Pollard, 2016). Another three studies evaluated the effects of exposure by the targeted application of triclopyr-2-butoxyethyl ester (Wojtaszek et al., 2005) and glyphosate (Thompson et al., 2004; Edge et al., 2014) pesticides in natural environments. The other studies related the effects on survival, morphology, and metabolism of tadpoles exposed to mixtures of pesticides present in water bodies adjacent to the study areas, such as agricultural crops (Sparling et al., 2014), such as soybean, maize, and sorghum (Agostini et al., 2020; Dick et al., 2021). Among the main pesticides determined in these environments, with greater effects on tadpoles are chlordane, endosulfan, chlorpyrifos, malathion, trifluralin, and chlorothalonil (Sparling et al., 2014), cypermethrin+glyphosate (Agostini et al., 2020), total neonicotinoids, atrazine and glyphosate (Dick et al., 2021).

When investigating the effects of the herbicide triclopyr-2-butoxyethyl ester on mortality, evasion response, and growth of tadpoles, Wojtaszek et al. (2005) used *in situ* enclosures in two humid forest areas and applied the herbicide in the environment. Sites were selected to compare wetlands, but with different depths, the presence of macrophytes, pH, and suspended sediments. The authors used 24 enclosures in the environment. Tadpoles of two native species (*Rana* [*Lithobates*] *clamitans* and *Rana* [*Lithobates*] *pipiens*) were studied and demonstrated that cumulative mortality was higher at higher treatment concentrations, with total mortality within the first 96 h after herbicide application. The interaction of the herbicide with biotic and abiotic factors is pointed out as the driver of the biological effects on the development, mobility, and survival of tadpoles (Wojtaszek et al., 2005). High pH has been described as increasing the toxic effect of pesticides (Chen et al., 2004), and physical, chemical, and biological characteristics of water can affect the persistence of these products in the environment (Wojtaszek et al., 2005). The insecticide cypermethrin, for example, in neutral and acid pH, remains stable; however, it tends to increase its hydrolysis when at alkaline pH (Laskowski, 2002). Some higher pH values (7.5) increase the toxic effects of herbicides on aquatic taxa (Chen et al., 2004). On the other hand, the decrease in pH can result in a delay in the rate of growth and development of tadpoles, which can affect the individual's body size and lower fitness (Beattie & Tyler-Jones, 1992).

Quantifying the probability and magnitude of glyphosate contamination, Thompson et al. (2004) monitored 51 wetlands using the species *Rana* [*Lithobates*] *pipiens* and *Rana* [*Lithobates*] *clamitans*. Areas were classified as overspray, adjacent, or buffered in relation to spray blocks. The physical-chemical sampling of these areas was performed by analyzing the water 48 hours after the herbicide application. Vegetation in buffered areas limited exposure to the herbicide, with a concentration ten times lower than in areas sprayed in excess. Factors related to sampling sites, such as differential interception by surrounding vegetation, degree of water surface occlusion by aquatic macrophytes, as well as the total surface and volume of the wetland are important for mitigating the range and effects of applied pesticides (Thompson et al., 2004). Edge et al. (2014) replicated, for two years, an experiment in Canada using six wetlands, where 24 small swamps were divided with an impermeable barrier. The authors tested exposure to glyphosate, applied alone and in combination with nutrient enrichment in amphibian survival and development.

Contrasting doses of glyphosate were used, with high concentration (2880 µg a.e./L) and low concentration (210 µg a.e./L). In parallel, a portion of the wetlands was maintained as a control, without the application of treatments. As a model species, the authors used *Rana sylvatica* [*Lithobates sylvaticus*] and *Rana* [*Lithobates*] *clamitans*. According to the authors, the nutrient enrichment associated with the application of glyphosate minimized the negative effects of glyphosate on tadpole abundance, making glyphosate less toxic to tadpoles. Although the results do not indicate the expected toxic effects of glyphosate on tadpoles in the field study, probably due to the short duration and mitigation of toxicity by environmental variables (Edge et al., 2014), positive effects of the herbicide on the abundance of *Rana* [*Lithobates*] *clamitans* (Edge et al., 2012) of concern as this species is able to completely remove other species from wetlands through egg mass predation (Edge et al., 2014). On the other hand, glyphosate formulations are widely studied under laboratory conditions and indicated as toxic to amphibians, especially in the larval stage, as reviewed by Gill et al. (2018). Furthermore, changes in swimming activity, damage to the mouth and intestine, and genotoxicity were recently observed in *ex situ* work by our research group on different tadpole species, such as *Physalaemus cuvieri* and *Physalaemus gracilis* (Herek et al., 2020; 2021), and *Boana faber* and *Leptodactylus latrans* (Pavan et al., 2021).

Recently, Agostini et al. (2020) worked in 91 temporary ponds in the Pampa Argentino, in a mixture of terrestrial environment and agricultural plots, sampling the tadpoles of the species *Boana pulchellus*; *Leptodactylus latrans*; *Rhinella fernandezae,* and *Rhinella Arenarum*. The testing proposal was the survival and mobility of tadpoles in these enclosures *in situ*, separating them by tanks, where each tank contained five enclosures and each enclosure with 20 tadpoles, exposed to different concentrations and combinations of pesticides. In parallel, eight ponds without pesticides, with 99.8% survival and without detection of effects on the mobility of tadpoles were used as controls. Among the ponds containing pesticides, the authors found greater toxicity with the treatment consisting of cypermethrin (124-354.9 ug/L detected) + glyphosate (18.2 – 320.7 ug/L detected), which caused a survival of less than 3 .5% and zero mobility after 48h exposure (Agostini et al., 2020). The synergy of factors, mainly abiotic, in the toxicity of pesticides has often been reported, where pH (Chen et al., 2004), and temperature (Camp & Buchwalter, 2016) can increase the toxic effects of pesticides by impacting the rate absorption, acting as a modulator of sublethal toxicity. This synergy is highlighted mainly in small lentic environments, where pesticide rates reach higher concentrations, compared to larger rivers and lakes (Agostini et al., 2020). According to these authors, although a greater impact on the mobility and survival of tadpoles is verified in 48 hours, these effects can be even more negative over time. In this survey, the way in which pesticides are used reflects the usual reality of application and use in South America, including southern Brazil, Argentina, and Paraguay (López et al., 2012) and, after applications in the fields, all the formulations reached high concentrations in the water columns, increasing the possibility of lethal and sublethal effects on tadpoles (Agostini et al., 2020).

In agricultural areas, several negative effects were observed *in situ* experiments on amphibian species. Attademo et al. (2014) evaluated the species *Scinax squalirostris* and *Leptodactylus mystacinus* in ponds maintained in rice plantation canals in Argentina, where the site received aerial spraying of the insecticide lambda-cyhalothrin. As a controlled environment, a preserved native area was used. The animals, in both natural environments, remained in perforated tanks, to allow the exchange of fluids with the environment, until they reached the Gosner stage 36-37, consisting of three replicates with 90 tadpoles per reproductive site. From the daily analysis, it was verified that the concentrations of nitrate and orthophosphate were higher and the levels of pH, dissolved oxygen, and conductivity were lower in the area of the rice crop in relation to the native environment. This may have contributed to the increased mortality and smaller size of tadpoles in agricultural areas (Attademo et al., 2014). The authors also verified the inhibition of acetylcholinesterase (AchE) activity and a reduction (25%) in carboxylesterase activity in tadpoles exposed to water courses used to irrigate these crops, which was not verified in the control areas. The variation in cholinesterases (AChE and BChE), which act in the hydrolysis of the neurotransmitter acetylcholine and in cholinergic synapses, and the activity of the glutathione-*S*-transferases enzyme, which acts in cellular detoxification (Khan et al., 2003; Araújo et al., 2016), associated with the decrease of mitotic erythrocytes in tadpoles kept in the agricultural area, are important biochemical markers of stress-inducing conditions (Barni et al., 2007).

The impact of agriculture on the tadpole species *Bufo bufo*, in England and Wales was evaluated by Orton & Routledge (2011). The authors obtained spawnings of *B. bufo* from four agricultural environments and kept them in tanks in the native environment (*in situ*). The *in situ* environments were classified as no or little contamination (reference), a site with little agricultural activity and two sites with high agricultural activity, and, consequently, greater use of pesticides. Subjects were analyzed over 15 weeks. The environments with low agricultural activity (69.5%) and with high agricultural activity (76.7 and 81.5%) showed high failure in hatching eggs (percentage of eggs that did not hatch), in relation to the reference environment (15 %). Still, one of the environments of high agricultural activity caused 100% of tadpole mortality (Orton & Routledge, 2011). Stress situations can raise corticosterone levels, and increase the unregulated metamorphic rate (Hayes, 2006), which can lead to the death of tadpoles. Compared to the *ex situ* experiment, all individuals kept in tanks, in the natural environment, had a smaller size, weighing up to 3.5 times smaller in the area with high agricultural activity (Orton & Routledge, 2011). Furthermore, under *in situ* conditions, greater sexual dimorphism was observed, being the first report of the intersex occurrence of European amphibian species.

More recently, Pollard et al. (2016) evaluated an area initially used for industrial purposes and landfills, with leachate contamination, which was remedied by a specific program in Sydney, Australia (Darcovich & O'Meara, 2008). For the remediation of the area, located in the Sydney Olympic Park, primary habitat areas of the bell frog (*Litoria aurea*) were structured consisting of 37 ponds in a corridor of 40 ha of land, in the 1990s (Darcovich & O'Meara, 2008; Pickett et al., 2013). Once the restructuring was carried out, *L. aurea* occupied most of the lakes (27 of the 33 lakes available in the 2000/2001 breeding season). However, occupancy gradually declined, with only seven of the 37 available lakes occupied in the 2011/12 breeding season. Pollard et al. (2016) found, when placing spawns in compartments in these lakes, the highest mortality rates occurred during the egg to embryonic stage of *L. aurea,* with some eggs showing no development. However, a similar survival rate of tadpoles was observed, as well as similar water quality of non-breeding ponds compared to breeding ponds, which may have allowed the high survival rate of *L. aurea* in these environments (Pollard et al., 2016). It is known that inadequate chemical water quality can hinder the development of embryos and prevent them from hatching amphibian eggs (Freda & Dunson, 1985).

In Argentina, water contaminated by the pesticide azinphosmethyl, determined in an irrigation channel for fruit orchards, showed negative effects on tadpoles of the native species *Rhinella arenarum* (Rosenbaum et al., 2012). According to the authors, the inhibition of AChE activity and carboxylesterase activity demonstrates the importance of using these neurotoxic biomarkers, allowing monitoring and risk assessment from the physiological and biochemical alteration of these organisms to chemical exposure.

Sparling et al. (2014) also evaluated the effect of pesticides in ponds on native frog populations. In Sierra Nevada lagoons, in the United States, the authors verified the presence mainly of malathion, trifluralin, and endosulfan, which were related to an increase in genotoxicity and reduced growth, development, and survival rates of *Pseudacris regilla* tadpoles.

In a similar study carried out in Canada, Dick et al. (2021) evaluated six areas of an irrigation channel for intensive agriculture, three in a dredged area and three in an unmanaged area. The authors verified that, with the presence of vegetation, the tadpoles of *Rana* [*Lithobates*] *pipiens* do not suffer harmful effects on survival, growth, and development, even with the relative increase of glyphosate in the environment after channel cleaning (Dyck et al., 2021). In graded/clean environments, with greater exposure to high temperature, it can be considered the most influential physicochemical parameter in the growth and development of tadpoles in dredged/cleaned areas, in relation to the lowest temperature observed in areas with vegetation/unmanaged (Camp & Buchwalter, 2016; Dyck et al., 2021). The increase in temperature can contribute to the desiccation of these environments in warmer periods of the year, eliminating the aquatic environment and threatening the survival of frogs if the terrestrial life stage has not yet been reached (Dyck et al., 2021). Amphibians are known to accelerate metamorphic development due to the risk of desiccation (Székely et al., 2017; Ruthsatz et al. 2018). However, anticipating metamorphosis may decrease the probability of survival also in the terrestrial adult stage (Altwegg & Reyer, 2003) and potentially decrease reproductive success (Rudolf & Rödel, 2007).

Although *in situ* experimentation is proving to be a faster and more realistic way to investigate the toxicological conditions of local environments (Gonçalves et al., 2019), more detailed descriptions and quantification of pesticides in the environment are still needed, in addition to more research that encompasses the entire amphibian life cycle, from aquatic to terrestrial stages (Dick et al., 2021). High pH can increase the toxic effect (Chen et al., 2004), and physical, chemical, and biological characteristics of the water can affect the persistence of pesticides in the environment (Wojtaszek et al., 2005). These broader and longer-term visions are important because of the interactions between pesticides and abiotic factors in the environment that can alter the toxicity of these chemicals and even the late development of amphibians. In addition, it is important, in parallel, to review the legislation for the use of pesticides, since numerous studies have already reported the toxic effects of different classes of pesticides in controlled experiments (*ex situ*) (Macagnan et al., 2017; Rutkoski et al., 2018, 2020, 2021; Wrubleswski et al., 2018; Vanzetto et al., 2019; Herek et al., 2020, 2021; Pavan et al., 2021), enabling better protection for the environment and also for the human species (Agostini et al., 2020) that are indirectly exposed to these contaminants. Relating population decline with the use of pesticides, *in situ*, is an arduous task and requires long monitoring of data in several regions accompanied by controlled experiments, since to understand the real impacts of pesticides on populations, more information is needed.

Final considerations

Available information on the interaction between native species and pesticides indicates the occurrence of malformations and structural damage, biochemical, genotoxic, and neurotoxic alterations, in addition to reduced growth and effects on development and survival in different amphibian species. Different species may present differential responses to exposure to pesticides. In addition, the concentrations of pesticides in the medium may vary depending on environmental factors, such as increasing temperature, changes in pH, and dissolved oxygen.

Even with a still incipient number of studies, the published works point to critical data mainly for the growth and development of amphibians. Continued long-term exposure can compromise the search and ingestion of food, and escape from predators, which will contribute to negative effects on the survival of these animals. Among the pesticides studied, there was a greater number of studies related to exposure to glyphosate, as it is one of the most used herbicides worldwide. However, the growing increase in the diversity of pesticides sold annually requires even more studies to avoid an alarming near-term population decline of the amphibian species.

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