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Water quality assessment and amphibian sensitivity in a protected area of the Atlantic Forest Biome

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ABSTRACT. The Atlantic Forest biome harbors exceptionally high biodiversity and endemism. Turvo State Park protects the largest remnant of Semideciduous Seasonal Forest in Rio Grande do Sul. Despite this biological richness, stressors such as pesticide contamination threaten species survival and development. Amphibians, due to their ecological and physiological traits, are particularly sensitive to environmental disturbances. In this study, we assessed the sensitivity of *Leptodactylus luctator* tadpoles in lentic environments located inside the park and within its buffer zone. Ten lentic habitats were evaluated (five in each area). Water samples from the 10 sites were analyzed for pesticide residues, and a spawn of *L. luctator* was collected from a lake inside the park. The spawn was reared in the laboratory until tadpoles reached Gosner stage 25, after which they were transferred to the study sites. After 14 days, tadpoles were measured, weighed, and examined for the presence of micronuclei (MN) and erythrocyte nuclear abnormalities (ENA). Water analyses revealed clomazone and chlorpyrifos inside the park, while atrazine, azoxystrobin, and imidacloprid were detected in the buffer zone. MN and ENAs—including binucleate, anucleate, notched, and lobed nuclei, nuclear buds, karyolysis, apoptosis, and micronuclei—were observed in tadpoles from both areas. These findings highlight the importance of biomonitoring in protected areas, particularly given the anthropogenic pressures exerted on their borders.

Keywords: Amphibian; Atlantic forest; pesticides; protected area; Turvo State Park.

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Introduction

Most of Brazil's agricultural production is located within the Atlantic Forest biome, where biodiversity and species endemism are concentrated primarily in forest fragments (Myers et al., 2000; Eisenlohr et al., 2015). Many of these fragments are protected under the Brazilian Forest Code (Brasil, 2012). However, they continue to suffer biodiversity loss, mainly due to human activities (Lima et al., 2020).

Turvo State Park, situated in the Atlantic Forest biome, protects 17,491.4 ha of forest and represents the largest forest fragment in Rio Grande do Sul (Sema, 2005). Thirty-two amphibian species have been recorded in and around the park, including 30 belonging to the order Anura, one from the order Gymnophiona, and one exotic species (Iop et al., 2011). The aquatic environments inside the park are surrounded by well-preserved forest, while the park's borders directly interface with agricultural areas, lacking a buffer zone to separate the protected ecosystem from crop production.

Native populations of anuran amphibians are particularly vulnerable to habitat degradation (Atkins et al., 2019). Their permeable skin increases susceptibility to environmental contaminants (Sigurdsen & Green, 2011; Larsen & Ramløv, 2013). Because amphibians rely on aquatic environments during early developmental stages, they are especially exposed to pollutants such as pesticides.

Pesticides are widely used in monoculture systems to control weeds and pests. However, many of these chemicals are leached and accumulate in natural aquatic environments (Pérez-Lucas et al., 2019; Riaz et al., 2021). In addition, contamination often results from the misuse of these products in agricultural areas (Agostini et al., 2020).

Amphibian population declines have been documented worldwide, with pesticide contamination identified as a major contributing factor (Attademo et al., 2014; Edge et al., 2014; Sparling et al., 2015; Agostini et al., 2020; Dyck et al., 2021). These compounds can impair tadpole development, reproduction, behavior, and morphology (Pavan et al., 2021; Macagnan et al., 2023, Santos et al., 2024). Genotoxic alterations, such as the formation of micronuclei and erythrocyte nuclear abnormalities (ENAs), have also been reported in amphibians exposed to pesticides (Rocha et al., 2020; Herek et al., 2021; Ascoli-Morrete et al., 2022; Samojeden et al., 2022). Owing to this sensitivity, amphibians are widely recognized as key bioindicators of environmental disturbances (Sparling et al., 2015; Hartmann et al., 2023).

Leptodactylus luctator (Hudson, 1892) a member of the family Leptodactylidae, is broadly distributed across tropical and subtropical regions of South America east of the Andes, including Argentina, Bolivia, Brazil, and Uruguay (Magalhães et al., 2020, Frost, 2024). The species is commonly found in ponds, small lakes, and flooded areas, with reproduction occurring in temporary waterbodies (IUCN, 2023). During the breeding season, eggs are deposited in floating foam nests on the water surface (Schvezov et al., 2023). Its conservation status is currently listed as Least Concern (LC) by the IUCN (2023), owing to its wide distribution and adaptability to diverse habitats.

This species has been extensively used as a bioindicator in laboratory studies, which have demonstrated its sensitivity to pesticides such as cypermethrin + glyphosate (Agostini et al., 2020), glyphosate + 2,4-D (Pavan et al., 2021), chlorpyrifos (Silva et al., 2021) imidacloprid (Samojeden et al., 2022), and S-metolachlor-based herbicide (Pereira et al., 2024). Exposure to these compounds has been shown to induce morphological, behavioral, and genotoxic alterations in *L. luctator* tadpoles (Pavan et al., 2021; Samojeden et al., 2022).

Despite substantial laboratory evidence, data on amphibian sensitivity under natural field conditions remain scarce. For instance, soil chemistry changes have been linked to alterations in the growth, development, and oxidative stress of *L. luctator* (Schvezov et al., 2023), while broader anthropogenic disturbances negatively affect overall health status (Bahl et al. 2024). *In situ* experimentation provides a rapid and realistic approach to evaluating the toxicological conditions of local environments (Gonçalves et al., 2019). However, more detailed methodological descriptions and concurrent quantification of environmental pesticide levels are necessary for a comprehensive understanding of their impacts on amphibians (Dyck et al., 2021). Here, we assessed the sensitivity of *L. luctator* tadpoles reared in ponds located in two distinct environments: the core and the buffer zone of Turvo State Park, an important protected area in southern Brazil.

Materials and methods

Study area

The study was conducted in Turvo State Park and its buffer zone (-27°13′57.58″ S, -53°51′04.58″ W). The park covers 17,491 ha and is located in the municipality of Derrubadas, southern Brazil, at an altitude ranging from 120 to 436 m above sea level. The forest physiognomy is classified as deciduous and extends into northeastern Argentina and western Paraguay (Instituto Brasileiro de Geografia e Estatística [IBGE], 2012).

Although the park contains a large preserved area, it is surrounded by agricultural land, with no effective buffer zone separating the forest from crop fields. The surrounding landscape matrix is composed mainly of soybean, corn, pastures, and mixed crops. For this study, areas located at least 500 m from the park edge were considered core areas, while areas outside the park were designated as the buffer zone.

Data collection was carried out in five ponds within the park's core area, where the surrounding matrix is preserved deciduous forest with a semi-closed canopy and an understory dominated by *Chusquea* sp. In the buffer zone, five ponds were selected within an agricultural production matrix lacking forest protection.

Physicochemical parameters of water

Water quality parameters were measured in both the core and buffer zone ponds using a multiparameter probe (HI9829, Hanna Instruments, Barueri, Brazil). Temperature, turbidity, ammonia, dissolved oxygen, pH, and conductivity were recorded on day 1 (when tadpoles were introduced), day 7, and day 14 of the experimental period.

Determination of pesticides

Pesticide analysis was performed at the Pesticide Residue Analysis Laboratory, *Universidade Federal de Santa Maria*. Solid-phase extraction with a polymeric solvent was used, followed by quantification with gas chromatography (GC-MS/MS) and liquid chromatography (LC-MS/MS) coupled to mass spectrometry. A standard set of 55 pesticide residues was screened, following Donato et al. (2015). The solid-phase extraction (SPE) and LC-MS/MS method had a detection limit (LOD) of 0.020 $\mu g\ L^{-1}$ and a quantification limit (LOQ) of 0.006 $\mu g\ L^{-1}$.

Test organism

A spawn of *Leptodactylus luctator* was collected in a well-preserved lentic environment within the park, away from the conservation unit's edge and nearby agricultural crops (Figure 1). The spawn, naturally deposited in water, was collected using plastic gloves and a sieve, then placed in a plastic box (41 × 29 × 14 cm) containing 8 L of dechlorinated, aerated water at room temperature. The eggs were incubated in the laboratory until reaching developmental stage 25 (Gosner, 1960). Tadpoles were then divided into 10 groups (3 groups of 15 per pond; 450 total). Each group was housed in cylindrical plastic pots (10 cm diameter × 15 cm height) and transferred to either core or buffer zone ponds. The species was selected because it is abundant both inside and around the park (Iop et al., 2011; 2012).

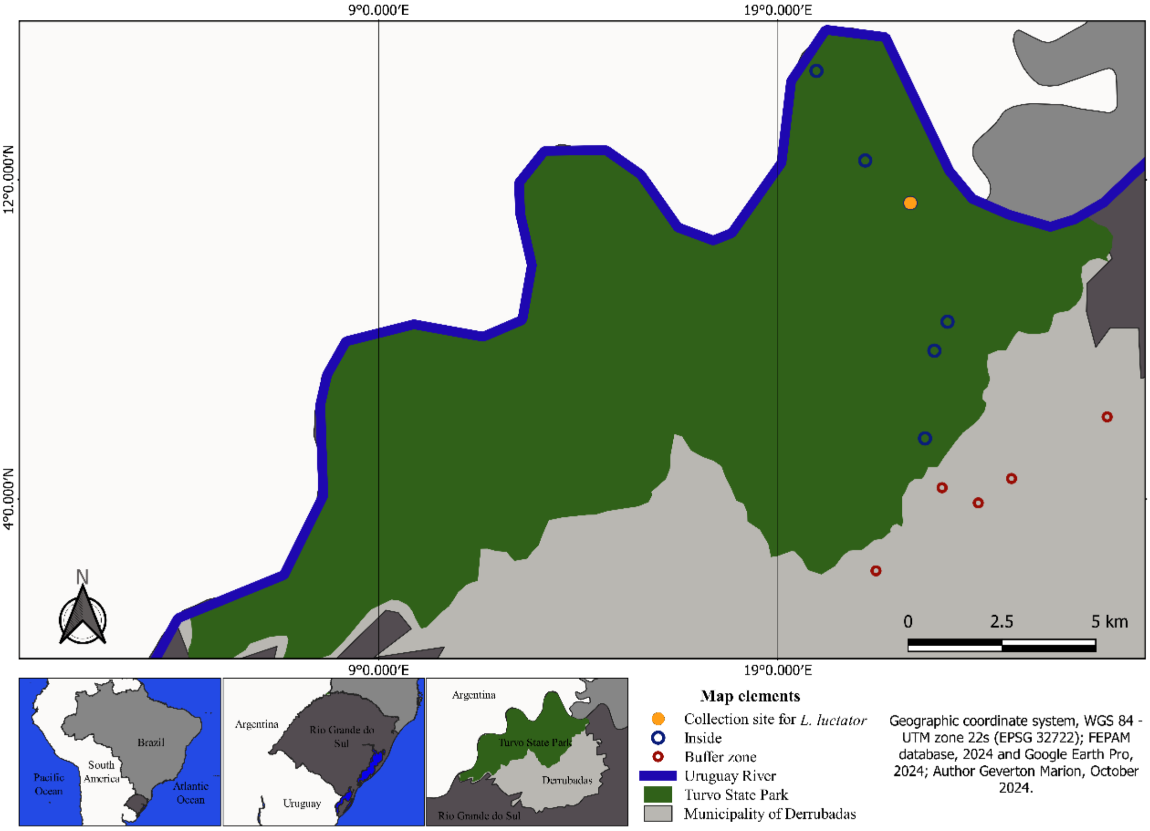

Figure 1. Location of ponds used for in situ experiments inside (core) and in the buffer zone of Turvo State Park, Derrubadas, RS, Brazil.

Experimental procedure

Five ponds inside the park (core) and five in the buffer zone were selected, with at least 500 m between them (Figure 1). Each pond received three groups of 15 tadpoles at Gosner stage 25, placed in cylindrical plastic traps (20 cm diameter × 30 cm height) covered with 1 mm mesh to allow water circulation. This totaled 45 tadpoles per pond. At the start of the experiment, 10 tadpoles were measured using a digital caliper (Absolute, Mitutoyo Corporation, Japan). Tadpoles in core ponds averaged 5.69 ± 0.97 mm (range: 3.5–7.5 mm), while those in buffer ponds averaged 6.05 ± 0.96 mm (3.8–7.9 mm), with no significant difference between groups ($U = 308$, $p = 0.19$). Tadpoles remained *in situ* for 14 days, with daily monitoring and *ad libitum* feeding with spinach. É preciso incluir o parecer do comitê de ética. Pedir aos autores.

Length and mass assessments

At the end of the 14-day period, all surviving tadpoles were counted. For morphometric analysis, excess water was removed with paper towels before measurement. Total length was recorded using a digital caliper (Absolute, Mitutoyo Corporation, Japan), and body mass was measured using an analytical balance (BM-A07, B-Max, China).

Erythrocyte nuclear abnormalities

For genotoxicity analysis, tadpoles were anesthetized with 2% lidocaine solution. Blood was collected with disposable heparinized insulin syringes and smeared onto sterilized microscope slides. Smears were fixed with

100% cold methanol (4°C) for 2 minutes and stained using the Panótico Rápido® kit (Laborclin Ltda, Pinhais, PR, Brazil). Slides were examined under an Olympus CX31® optical microscope (Tokyo, Japan) at 100× magnification.

ENAs were scored by evaluating 1,000 erythrocytes per tadpole, Carrasco et al. (1990) and Fenech et al. (2011). Abnormalities recorded included: (1) anucleated cells; (2) apoptotic cells (fragmented nuclei); (3) binucleated cells; (4) nuclear buds (evaginations similar in size to micronuclei); (5) karyolysis (nuclear outline without internal material); (6) notched nuclei (slit or indentation, kidney-shaped); (7) lobed nuclei (large evaginations); and (8) micronuclei. Micronuclei were identified using three criteria (Pérez-Iglesias et al., 2015): (a) no overlap with the main nucleus, located in the cytoplasm; (b) staining intensity equal to the main nucleus; (c) rounded shape with no connection to the main nucleus. Results were expressed as ‰ (per 1,000 cells).

Statistical analysis

All data were analyzed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). Variance homogeneity was tested with Levene's test, and normality with the Shapiro–Wilk test. As assumptions of homogeneity and/or normality were not met, nonparametric Mann–Whitney tests were applied. Statistical significance was set at $p < 0.05$.

Results

Physicochemical parameters of water

The physicochemical parameters of the water differed between environments, with lower values in the core compared to the buffer zone (Table 1). Temperature (U = 46.5; p = 0.005), turbidity (U = 58.0; p = 0.005), ammonia (U = 2.5; p = 0.04), and dissolved oxygen (U = 36.0; p = 0.0002) differed significantly between the two environments. In contrast, pH (U = 68.0; p = 0.06) and conductivity (U = 69.5; p = 0.07) showed no significant differences (Table 1).

Table 1. Physicochemical parameters of water from ponds in core and buffer zones of the Turvo State Park, Derrubadas, RS, Brazil.

Parameters	Core	Buffer
pH	6.47 ± 0.31 (5.86 – 6.87)	6.68 ± 0.14 (6.36 – 6.87)
Conductivity (S m^{-1})	43.73 ± 28.89 (12.00 – 83.00)	64.53 ± 13.87 (48.00 – 86.00)
Temperature (°C)	20.76 ± 1.40 (18.90 – 23.00)	22.50 ± 1.41 (20.50 – 24.00)*
Turbidity (UNT)	9.06 ± 9.39 (0.50 – 29.00)	13.79 ± 2.44 (9.90 – 17.10)*
Dissolved oxygen (mg L^{-1})	0.02 ± 0.07 (0.00 – 0.27)	2.20 ± 2.29 (0.00 – 5.96)*
Ammonia (mL)	0.25 ± 0.00 (0.25 – 0.25)	0.65 ± 0.33 (0.25 – 1.00)*

Means ± standard deviation (n = 5). Values in parentheses indicate minimum and maximum values. Asterisk (*) indicates a significant difference ($p < 0.05$) between the core and buffer zones of the park, by the Mann-Whitney test. Measurement unit: Siemens per meter (S m^{-1}), degrees Celsius (°C), nephelometric turbidity units - UNT, milligrams per liter (mg L^{-1}).

Determination of pesticides

Residues of clomazone and chlorpyrifos were detected in core ponds within the park. In the buffer zone, atrazine (below the method detection limit), azoxystrobin (0.022 µg L^{-1}), and imidacloprid (0.028 µg L^{-1}) were detected (Table 2).

Table 2. Pesticides analyzed in core and buffer zones of Turvo State Park, Derrubadas, RS, Brazil.

Pesticide	Core	Buffer Zone
Atrazine	n.d.	< LOQ
Azoxystrobine	n.d.	0.022
Clomazone	< LOQ	n.d.
Chlorpyrifos	< LOQ	n.d.
Imidacloprid	n.d.	0.028

* n.d = Not Detected; ** < LOQ = Limit of quantification

Survival and body size

At the end of the *in situ* experiment (14th day), from the 225 individuals placed in each environment, 20 tadpoles survived in the buffer zone ponds. The survival percentage did not differ between the areas evaluated (U = 7.0; p = 0.27; Table 3).

Tadpole length was 10.67 mm (core ponds) and 14.06 mm (buffer ponds) (Table 3). Tadpoles in core ponds had significantly smaller total length (U = 164.0; p = 0.01) compared to those in buffer ponds.

Table 3. Survival, total length and mass of *Leptodactylus luctator* tadpoles in core and buffer zone environments at Turvo State Park, RS, Brazil.

Parameters	Core	Buffer
Survival (%)	8.89 ± 13.05 (0.00 – 28.89)	17.33 ± 7.59 (6.67 – 24.44)
Total length (mm)	10.67 ± 2.53 (6.73 – 14.70)	14.06 ± 4.84 (4.02 – 28.09)
Mass (g)	0.88 ± 0.08 (0.70 – 1.03)	1.01 ± 0.14 (0.73 – 1.50)

Means ± standard deviation (n = 5). Values in parentheses indicate minimum and maximum values.

Analysis of erythrocyte nuclear abnormalities (ENA)

Micronuclei and ENAs—including anucleated cells, binucleated cells, notched nuclei, nuclear buds, lobed nuclei, karyolysis, and apoptosis—were observed in both environments (Table 4; Figure 2). No significant differences were detected in micronuclei frequency (U = 389.5; *p* = 0.89) or in total ENA frequency between zones. However, karyolysis occurred significantly more often in the buffer zone compared to the core (U = 225.0; *p* = 0.004; Table 4).

Table 4. Erythrocyte Nuclear Abnormalities (ENAs) and micronuclei in *Leptodactylus luctator* tadpoles from core and buffer zone of Turvo State Park, Derrubadas, RS, Brazil. Frequency of abnormalities is expressed in ‰.

Parameters	Core	Buffer zone
Micronuclei	0.05 ± 0.22 (0.00 – 1.00)	0.05 ± 0.22 (0.00 – 1.00)
Anucleated cells	2.80 ± 1.73 (0.00 – 6.00)	3.43 ± 2.24 (0.00 – 8.00)
Binucleated cells	1.05 ± 0.94 (0.00 – 3.00)	1.41 ± 1.27 (0.00 – 5.00)
Notched nucleus	0.85 ± 1.13 (0.00 – 4.00)	0.89 ± 1.23 (0.00 – 5.00)
Nuclear bubble or bud	0.45 ± 0.75 (0.00 – 2.00)	0.79 ± 1.17 (0.00 – 4.00)
Lobed nucleus	0.35 ± 0.74 (0.00 – 2.00)	0.58 ± 0.96 (0.00 – 4.00)
Karyolysis	0.35 ± 0.74 (0.00 – 2.00)*	1.10 ± 1.09 (0.00 – 4.00)
Apoptosis	4.95 ± 1.76 (2.00 – 9.00)	4.23 ± 2.96 (0.00 – 10.00)
Number of cell analyzed	20.000	39.000
Total ENAs (‰)	1.08	1.25

Means ± standard deviation (n = 5). Values in parentheses indicate minimum and maximum values. Asterisk (*) indicates a significant difference (*p* < 0.05) between core and buffer zones (Mann-Whitney test).

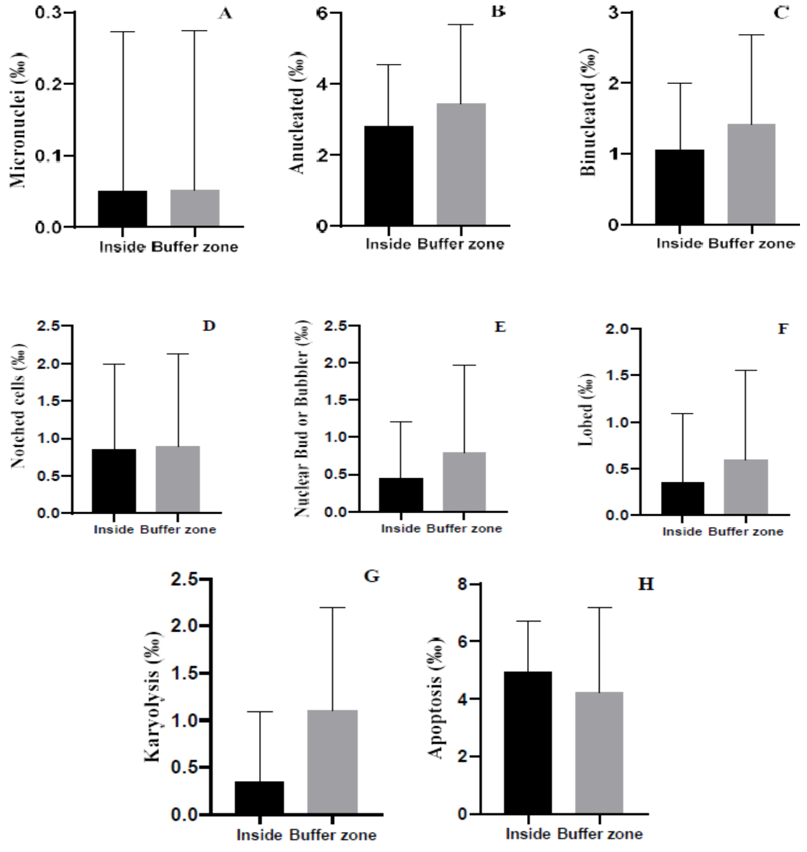

Figure 2. Micronuclei and nuclear abnormalities in erythrocytes of *Leptodactylus luctator* tadpoles from Turvo State Park, Derrubadas, RS, Brazil. (A) Normal cell; (B) Micronucleus; (C) Anucleate cell; (D) Binucleate cell; (E) Notched nucleus; (F) Nuclear bud; (G) Lobed nucleus; (H) Karyolysis; (I) Apoptosis. Bars represent mean ± SD (n = 15). Asterisks (*) indicate significant differences (p < 0.05) between core and buffer zones (Mann–Whitney test).

Discussion

Here, we show that tadpoles exhibited low survival rates and cytotoxic effects. In addition, we detected differences in water quality between environments, as well as the presence of pesticide residues in both the core and buffer zones of the park.

Survival was extremely low, consistent with previous laboratory studies that demonstrated the species' sensitivity to pesticide toxicity (Agostini et al., 2020; Pavan et al., 2021; Silva et al., 2021; Samojeden et al., 2022). Our results further demonstrate that this group is also sensitive to changes in natural habitats (lentic environments). Amphibians are particularly vulnerable to environmental change, especially pollution and habitat fragmentation, because they live at the interface of terrestrial and aquatic systems and can easily absorb pollutants through their skin. This makes *in situ* experiments complex but highly valuable for assessing habitat susceptibility.

Contrary to expectations, we observed higher mortality in the core ponds and greater survival in the buffer zone. The higher survival of tadpoles in buffer zone ponds may be associated with *L. luctator* being a generalist species better adapted to open environments (Brito & Costa, 2022). Generalist species often persist and even dominate in agricultural landscapes (Saccol et al. 2022). Another possible explanation is that the experiment took place in late March, coinciding with the end of the species' reproductive season in the region, meaning the spawn used may have represented the last cohort of the year.

Among surviving tadpoles, those developing in buffer zone ponds showed greater growth in both length and mass. As ectotherms, amphibian metabolism depends on environmental temperature (Wells, 2007). Higher temperatures may have stimulated increased movement and feeding, promoting growth. Braga and Lima (2001) found that the exotic species *Lithobates catesbeianus* had greater food consumption and weight gain at higher temperatures. In contrast, native species such as *Pleurodema diplolister* and *Rhinella granulosa* showed divergent responses at 30°C, with *P. diplolister* tadpoles exhibiting reduced growth while *R. granulosa* increased in size (Maciel & Juncá, 2009). Moreover, interactions between temperature and pesticides may have influenced body size, as shown by Grott et al., (2022), where tebuthiuron affected *L. catesbeianus* metamorphosis differently at 25°C (accelerated) versus 32°C (delayed with mass loss). *Rana arvalis* tadpoles also displayed reduced activity at acidic pH but elevated corticosterone in acid-adapted populations (Scaramella et al., 2022), indicating species-specific plasticity to water chemistry.

Water quality differed between zones, with core ponds showing lower temperature, turbidity, and dissolved oxygen compared to buffer ponds. Pesticides were detected in small quantities but were more frequent in the buffer zone. Although concentrations were low, residues were identified in both environments, including azoxystrobin (0.022 μg L^{-1}) and imidacloprid (0.028 μg L^{-1}) in the buffer zone. A previous study of park streams recorded 28 pesticides across different lakes (Rocha et al., 2020). Those authors noted that forest cover reduced pesticide influx from adjacent crops by 2.7-fold, consistent with our findings; residues inside the park were observed but below detection limits. Unlike Rocha et al. (2020), we detected azoxystrobin and imidacloprid only in ponds, not streams—an inconsistency that warrants further study.

Azoxystrobin, a strobilurin-class fungicide (Agrolink, 2022), inhibits mitochondrial respiration, reducing oxidative phosphorylation and ATP production (Koehler & Shew, 2018). Imidacloprid, a neonicotinoid, selectively targets insect nicotinic acetylcholine receptors, disrupting synaptic transmission (Di Muccio et al., 2006), and causing neurological effects or death (Mikolić & Karačonji, 2018). It is classified as a Group E carcinogen (no human carcinogenicity evidence; Epa, 2003). The low pesticide levels detected in lentic environments may reflect the off-season timing of the experiment, with long intervals since the last pesticide applications in surrounding agricultural fields.

Another aspect evaluated was erythrocyte nuclear abnormalities. Micronuclei (MNs) and other ENAs were detected in tadpoles from both core and buffer ponds, though no statistically significant differences were observed between environments. MNs are small intracytoplasmic chromatin fragments resulting from chromosomal breaks or whole chromosomes displaced by mutagenic agents (Gauthier et al., 1993; Lajmanovich et al., 2005). Their formation reflects errors during anaphase, where acentric chromatid fragments (clastogenic effect) fail to integrate into daughter cell nuclei due to spindle missegregation (Obiakor et al., 2012). Additional causes include DNA repair errors, chromosome segregation failures, and mitotic spindle dysfunction, leading to weak chromosomal attachment (Fenech et al., 2011). Thus, MN frequencies are widely accepted biomarkers of chromosomal damage (Norppa & Falck, 2003; Nüsse et al., 1996; Lajmanovich et al., 2005). MN occurrence has been extensively reported in pesticide-exposed amphibians, including *Hyla pulchella* exposed to endosulfan (Lajmanovich et al., 2005), and*L. luctator* exposed to glyphosate+2,4-D (Pavan et al., 2021) and imidacloprid (Samojeden et al., 2022).

Rocha et al. (2020) also analyzed micronuclei formation in tadpoles in natural environments. They studied *Boana curupi* and *Crossodactylus schmidti* in streams and linked chromosomal damage to both pesticides and ultraviolet (UV) radiation. The number of micronuclei in those species was similar to what we observed in *L. luctator* in this study. It is possible that MN formation is more strongly related to UV exposure than pesticide residues, since our *L. luctator* tadpoles were shielded from direct UV radiation, unlike *B. curupi* and *C. schmidti*. Because few studies evaluate multiple causal factors, more in situ research with amphibians is needed to clarify drivers of chromosomal damage.

Other ENAs observed also provide insight into DNA integrity in amphibians. Cells with cytokinesis block result in binucleated forms (Çavas & Gozukara, 2005). Nuclear buds resemble micronuclei (Prieto et al., 2008) and appear as nuclear bubbles with similar cytomorphology (Borges et al., 2019). Lobed nuclei may be precursors to MN formation (Harabawy & Mosleh, 2014), while anucleate cells may represent an adaptation that increases oxygen transport efficiency in polluted waters (Glomski et al., 1997, Barni et al., 2007). Apoptosis plays a regulatory role in tissue development and homeostasis (Kiechle & Zhang, 2002) and notched nuclei represent abnormal nuclear vacuoles of appreciable depth (Arcaute et al., 2014).

Cellular damage in amphibians may arise from several external stressors, including habitat fragmentation (Cushman, 2006). Micronucleus formation is mainly associated with environmental variation or contaminant exposure (Fenech, 2003). The occurrence of MNs and ENAs is therefore important for assessing chromosomal damage that may ultimately lead to cell death in amphibians (Pérez-Iglesias et al., 2015; Schuch et al., 2015; Getelina et al., 2022). Although ENA frequencies in L. luctator did not differ significantly between core and buffer ponds, these results are valuable as baseline data for future comparisons and for guiding experimental designs investigating the effects of fragmentation and/or pesticide contamination on amphibians.

The number of studies on pesticide effects in amphibians has grown, but in situ research on toxicological conditions in lentic environments remains limited. As demonstrated here, parameters such as temperature and other physicochemical characteristics of water can strongly influence amphibian development. Although no significant pesticide effects were observed on survival or genotoxic alterations in L. luctator, our results highlight that both water quality parameters and species-specific traits are crucial considerations in in situ experiments. Genotoxic alterations were recorded in tadpoles both inside the park and in its buffer zone.

We emphasize the need for further biomonitoring studies in protected areas and their surroundings, including both generalist and specialist species, to evaluate how preserved versus anthropized environments affect amphibian development. Such research is essential to assess species health and habitat conservation status. Only through comprehensive monitoring can we inform regulations and policies that effectively preserve these critical habitats, which are fundamental for biodiversity conservation.

Conclusion

We detected the presence of pesticides both inside and around Turvo State Park, with clomazone and chlorpyrifos found within the park, and atrazine, azoxystrobin, and imidacloprid detected in the buffer zone. Tadpoles from both areas exhibited nuclear abnormalities (MN and ENAs), including micronuclei, binucleated cells, karyolysis, and other nuclear alterations, indicating genotoxic and cytotoxic effects associated with pesticide exposure. These findings highlight the infiltration of pollutants into conservation areas and their potential impacts on amphibian health, even within protected environments. Given the ecological sensitivity of amphibians and their role as bioindicators, continuous biomonitoring is essential to assess the long-term effects of agricultural contaminants. Furthermore, stricter regulations on pesticide use near protected areas and improved buffer zone management strategies should be implemented to mitigate risks to biodiversity.

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