

Anurofauna in an Impacted Area in the Municipality of Ituverava in the São Paulo State of Brazil

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Abstract: There is great concern about the world amphibian populations reduction resulting from six major ecological factors cited as causes of modern amphibian declines and extinctions. Habitat loss resulting from deforestation and pollution of water reservoirs are usually events in agricultural production processes in Brazil and are important examples of these factors. In addition, there are many gaps in the knowledge of amphibian communities across the Brazilian territory. We carried out an anurofauna inventory for an impacted rural area, consisting of a pond located in the middle of an agricultural soybean plantation, in the municipality of Ituverava, State of São Paulo, in 2009. The inventory showed 15 species of anurans in 4 families. The most abundant families were Hylidae (six species) and Leptodactylidae (six species), one of them belonging to the subfamily Leiuperinae. The families Bufonidae (two species) and Microhylidae (one species) were also observed. Two species identified could be interesting finds, since the registration of *Pseudis bolbodactyla* (Lutz, 1925) in the São Paulo state until the present moment has no mentioned, and *Rhinella mirandaribeiroi* (Gallardo, 1965), was first described recently to São Paulo state in the municipality of São Joaquim da Barra, 30 km distant for the study area. The species was well adapted to their microhabitats, even with high ecological degradation, showing themselves to be opportunistic and tolerant to anthropic areas. Unexpectedly the pond started to dry, and it dried up totally in the second half of 2010. Then a new inventory was done in the study area for raining season in 2010, showing 6 species. The species were in 4 families (one for Hylidae, one for Leptodactylidae, one for Bufonidae, and three for Microhylidae), all showing explosive reproduction behavior in temporary pools formed by heavy rain. The study data stayed archived by twelve years. To conclude this work, the environmental conditions of the study area were analyzed in the year 2021. The pond region was substituted by a pasture for cattle confinement. We couldn't observe even tadpoles in temporary puddles in periods of torrential rain. The soil is compacted with large gullies. Unfortunately, all species inventoried in 2009 and 2010 could not be found. In that way, this study highlights the worrying reality of environmental degradation involving agriculture and its relationship with anurofauna in Brazil.

Keywords: Anthropogenic Tolerant Species, Anuran Inventory, Environmental Degradation, Impacted Area, Temporary Ponds

1. Introduction

Amphibians are a diverse vertebrate group with 8,393 species worldwide described until November 2021 [1]. Brazil has the highest diversity of amphibians in the world, comprising 1,188 species, with 1,144 anurans, 39 caecilians and 5 salamanders [2]. Many herpetologists began to observe a worldwide decline in amphibian populations from the

second half of the 1980s onwards [3]. The reference [4] cited six major causes of modern amphibian declines and extinctions: (1) commercial use; (2) introduced exotic species that prey on, compete with, and parasitize native species; (3) land-use change; (4) contaminants; (5) climate change; and (6) infectious disease. Reference [5] published a revision about Brazilian amphibian's decline, showing that it can be associated with habitat loss, interspecific interactions,

natural fluctuations, or lack of intensive sampling. This study indicates possible declines within the Cerrado biome, montane, and stream-associated populations. Reference [6] found no evidence for the amphibian decline in abundance or biomass in the Central Amazon Forest, over 22 years of analyzing the leaf litter. But the situation is not the same for the Brazilian Atlantic Forest, home to over 600 amphibian species [7] and one of the most threatened environments of the world [8]. Reference [9] provide strong evidence that chytridiomycosis caused most historical amphibian declines observed in Brazil across the Atlantic Forest. A recent study showed significant reductions in species abundance and species richness of amphibians in the Atlantic forest, especially for climate changes [10]. Many authors observed the decline or extinction of anuran populations in some areas of the southeastern Atlantic Forest, including Santa Tereza, Espírito Santo [11], Serra do Japi [12], Boracéia in São Paulo [13, 14]. For certain biomes in Brazil, such as the Atlantic Forest, population declines or even amphibian extinctions have been attributed to deforestation [15]. Brazil has been recognized as a global food producer, especially as a BRIC member [16], but the agricultural processes are resulting in habitat losses and pollution of water reservoirs for local anurofauna [17, 18]. In addition, there are many gaps in the knowledge of amphibian communities across the Brazilian territory. Seeking to obtain a better understanding of the diversity of anurofauna and their relation with ecologically disturbed environments, an anuran inventory was carried out for an impacted rural area, consisting of a pond located in the middle of an agricultural soybean plantation, in the municipality of Ituverava in the São Paulo State of Brazil, in 2009.

2. Methodology

2.1. Study Area

The municipality of Ituverava, located in São Paulo state, has an area of 727 km², situated 631 m above sea level, in the geographic coordinates at latitude 20°20'30" S and longitude 47°47'30" W. Its location on the map is in the northeast State of São Paulo, São Paulo Western Plateau, located in southeastern Brazil. The soil is composed of red soil and sandy originated from the decomposition of volcanic basalt, which makes it an excellent place for agriculture, especially grain crops, sugar cane, cotton, among other crops. The native vegetation was practically extinguished by agricultural practice, appearing in only small and rare patches. The climate is predominantly tropical seasonal with two well-defined seasons: the hot and rainy summer (October to March or April) and more mild and dry winter [19]. The municipality belongs to an area characterized by relief of "cuestas", belonging to the sedimentary basin Parana River. The study area is located at a private farm in Ituverava at latitude 20°20'47" S and longitude 47°48'09" W with an area of 3.0 ha. It was constituted in 2009 by a permanent pond (with a surface of 1.5 ha) surrounded by two types of vegetation (1) a natural gallery forest, very

fragmented, and (2) a monoculture of soybeans (Figure 1). In the past this vegetation was a mixture of Atlantic Forest, Cerrado, Cerradão, and the Riparian Forest [20]. The water's pond seems to come from a large pipe and from the annual rainfall.

2.2. General Information About the Inventory

Our objective was to inventory the anurofauna in the study area in 2009, and we did. But even finished the inventory, we noted that the pond was drying. Then it dried up in the second half of 2010, and we decided to inventory the study area for raining season in that year. The study data stayed archived by twelve years. Thinking about a way to conclude this study and thus publish these data, we decided to analyze the general environmental conditions of the study area in 2021 and monitory species in the rainy season.

2.3. Data Collection

For this inventory, a data collection methodology based on the previous study [21] was used. In 2009, the expeditions to the study area were carried out biweekly, between 18:00 and 00:00 hours, between February and December. In 2010, those were in the same way between October and December. In 2021 we couldn't find any species. Specimens were observed, photographed, and identified at first sight from occasional encounters in the field. We identified the specimens according to their morphology from updated and specialized literature. We collected testimony specimens and transported them in plastic bags to the laboratories of the Faculdade de Filosofia, Ciências e Letras de Ituverava. They were euthanized with 10% lidocaine, fixed in 10% formalin and preserved in 70% alcohol. Then they were deposited as samples in the herpetological collection at Anuran Ecology and Behavior Laboratory, Department of Animal Morphology and Physiology, Universidade Estadual Paulista, Jaboticabal, State of São Paulo, Brazil, under curation by Cynthia Peralta de Almeida Prado (CPAP). We used a scientific collecting permit issued by ICMBio (1015-3).

3. Results and Discussion

The inventory did in 2009 showed 15 species of anurans in 4 families. The most abundant families were Hylidae (with six species) and Leptodactylidae (with six species, one of them belonging to the subfamily Leiuperinae). We observed too the families Bufonidae (two species) and Microhylidae (one species). The inventory did in 2010 showed 6 species when compared with the 2009 inventory. The species were in 4 families (one for Hylidae, one for Leptodactylidae, one for Bufonidae, and three for Microhylidae), all showing explosive reproduction behavior in temporary puddles formed by heavy rains. In 2021 we couldn't find any species in the study area. All species inventoried in 2009 and 2010 are presented in Table 1.



Figure 1. A. Panoramic view of the study area showing the pond and the soybean monoculture (pointed by a red arrow) in 2009. B. Approximate view of the pond showing the aquatic vegetation. The yellow arrow shows the soybean monoculture a few meters from the shore. C. Aquatic and terrestrial vegetation surround the pond; D. Monoculture beside the pond. The blue arrow shows the pond.

Table 1. Anuran families inventoried in the study area in 2009 and 2010.

Families and Species	Inventory year	Microhabitat*	IUCN 2021**
Hylidae			
<i>Dendropsophus minutus</i> [22]	2009	2	LC
<i>Boana albopunctata</i> [23]	2009	2	LC
<i>Pseudis bolbodactyla</i> [24]	2009	3	LC
<i>Scinax fuscomarginatus</i> [24]	2009	2	LC
<i>Scinax fuscovarius</i> [25]	2009	1,5	LC
<i>Scinax cf. similis</i> [26]	2009	1,5	LC
<i>Trachycephalus typhonius</i> [27]	2010	1,3,6	LC
Leptodactylidae			
<i>Leptodactylus fuscus</i> [40]	2009	1	LC
<i>Leptodactylus labyrinthicus</i> [23]	2009	1	LC
<i>Leptodactylus latrans</i> [41]	2009	1,3,5	LC
<i>Leptodactylus mystacinus</i> [42]	2009	1,3	LC
<i>Leptodactylus podicipinus</i> [43]	2009	1,5	LC
<i>Physalaemus cuvieri</i> [44]	2009	1	LC
<i>Physalaemus nattereri</i> [45]	2010	1,3	LC
Bufonidae			
<i>Rhinella diptycha</i> [56]	2009/2010	1,3,6	DD
<i>Rhinella mirandaribeiroi</i> [57]	2009	4	LC
Microhylidae			
<i>Elachistocleis cf. cesarii</i> [60]	2009/2010	1	----
<i>Chiasmocleis albopunctata</i> [61]	2010	1,6	LC
<i>Dermatonotus muelleri</i> [61]	2009	1,3,6	LC

* The species were observed in the following microhabitats: on the banks of the pond (1), in the vegetation (2), in the pond water (3), in the objects in the pond (4), on the ground (5), puddles formed by rains (6). ** The red list categories of IUCN: Least Concern (LC) and Deficient Data (DD).

3.1. Family Hylidae

The family Hylidae was represented by six species in the 2009 inventory: *Dendropsophus minutus* [22]; *Boana albopunctata* [23]; *Pseudis bolbodactyla* [24]; *Scinax fuscomarginatus* [24]; *Scinax fuscovarius* [25]; *Scinax cf. similis* [26]. One species was registered in the 2010 inventory: *Trachycephalus typhonius* [27].

(1) *Dendropsophus minutus* (Figure 2A) was one of the most abundant species in the study area, and we observed it perched on the vegetation, above and around the pond vocalizing. We observed that males showed intense spikes of reproductive vocalizations for almost every month of the year, except for the coldest days of June when the air temperature reached 4°C. These data corroborate studies by reference [28], who showed the occurrence of testicular activity in males of *D. minutus* in the colder months, where they frequently vocalized. *Dendropsophus minutus* is a species that is also very adaptable to the anthropic environment. It is in the IUCN Red List Least Concern category, is not currently threatened, with stable populations. It occurs throughout Brazil, in open areas in the Atlantic Forest, in the Cerrado and Pantanal, up to 1,800 m altitude, being one of the most common anuran species in South America [29].

(2) *Boana albopunctata* (Figure 2B) was observed perched on shrubby vegetation and in the ground around the pond, starting in June, a gradual reduction in its vocalizations even in the wettest months. Despite the studies by reference [30] record reproductive activities of *B. albopunctata* during the rainy season, the species presented its predominant vocalization peaks in March, April, and May, which were drier periods. The species is in the IUCN Red List Least Concern category, not threatened, and is distributed in stable populations. The species is commonly observed in anthropogenic areas, showing tolerance to the degradation of its habitat, being distributed throughout the central, southern, and southeastern regions of Brazil, in the Atlantic Forest, Cerrado, and Pantanal, including the state of Rondônia, in up to 2,000 m of altitude [31].



Figure 2. A. *Dendropsophus minutus*. B. *Boana albopunctata*.

(3) *Pseudis bolbodactyla* (Figure 3A) was observed floating in the pond superfices. They are anchoring themselves to the vegetation in high densities with vocalization peaks between February to April and from

August to December. Although there are no consistent reproductive data regarding *P. bolbodactyla*, studies [32] for *Pseudis minuta* showed that the vocalization periods for this species were from October to March of the following year. Our data corroborated the references [33] and [34] about the general biology of *P. bolbodactyla*, showing that it is hugely dependent on water bodies, with several morphologic, and developmental adaptations to aquatic environments. The species is in the IUCN Red List Least Concern category, with not sufficient data on their population trend. Domestic livestock and water pollution from agriculture are threats for this species. This species has been found in some southern regions of Brazil, as Minas Gerais, south part of Goiás, south Bahia, Mato Grosso and the northern part of Espírito Santo, living in the Cerrado, Caatinga and Atlantic Forest domains, from sea level up to 1,000m [35].

(4) *Scinax fuscomarginatus* (Figure 3B) was observed in low vegetation around the pond, with peaks of vocalization in the rainy months. It is the IUCN Red List Least Concern category, is not currently threatened, adapting to anthropogenic disturbance. It is a typical species of open areas such as the Pantanal, in the Cerrado, and can also be found in the Atlantic Forest. It is distributed throughout the central region of Brazil, up to 2,000 m in altitude [36].



Figure 3. A. *Pseudis bolbodactyla*. B. *Scinax fuscomarginatus*.

(5) *Scinax fuscovarius* (Figure 4A) was observed on the ground among the undergrowth on the banks of the pond between September and December, showing explosive reproduction with its peak of male vocalization in September. It is a common species in urban areas, even being able to take shelter in homes. It is in the IUCN Red List Least Concern category, not threatened, and its populations are currently stable. It is distributed throughout southern, southeast and central Brazil, being observed in open areas in the Atlantic Forest, in the Cerrado, and Pantanal, up to 2,000 m in altitude [37].

(6) *Scinax cf. similis* (Figure 4B) was observed on the ground among the undergrowth on the banks of the pond between September and December in reproductive activity concomitant with *S. fuscovarius*, showing also explosive reproduction. It is a species that easily adapts

to the anthropic environment, and can be found inside homes in rural areas and in artificial open areas. It is in the IUCN Red List Least Concern category, is not under threat, and its populations are currently stable. It is an endemic species to Brazil, occurring in the coastal region of Caraguatatuba, in the State of Rio de Janeiro and in other areas of the Southeast, in the Atlantic Forest and in the Cerrado, being observed in open areas, lowlands, swamps, Restinga vegetation, pastures, secondary forests degraded and lakes, up to 1,100 m of altitude [38].



Figure 4. A. *Scinax fuscovarius*. B. *Scinax cf. similis*.

- (7) *Trachycephalus typhonius* (Figure 5A) was observed vocalizing during the nights in temporary puddles formed on the grass by heavy rains from October to December, showing explosive reproduction. The species is in the IUCN Red List Least Concern category, is not under threat, and its populations are currently stable. As it adapts easily to disturbed environments, it can live in second-growth forests, plantations, and human habitations. It has a wide distribution throughout the Brazilian territory, found in the Atlantic Forest, Cerrado, Pantanal, and throughout the Amazon basin, up to 800 m in altitude. With nocturnal habits, it can be found perching on tree branches, in bromeliads, in holes and under tree bark, and in the sheaths of banana trees during the dry season [39].

3.2. Family Leptodactylidae

The family Leptodactylidae was represented by six species in the 2009 inventory: (1) *Leptodactylus fuscus* [40]; (2) *Leptodactylus labyrinthicus* [23]; (3) *Leptodactylus latrans* [41]; (4) *Leptodactylus mystacinus* [42]; (5) *Leptodactylus podicipinus* [43]; (6) *Physalaemus cuvieri* [44]. One species in the 2010 inventory was registered: (7) *Physalaemus nattereri* [45]. Two species belong to the subfamily Leiuperinae.

- (1) *Leptodactylus fuscus* (Figure 5B) was observed in burrows between the grass and the banks of the pond, presenting their peak of vocalization in the rainy season, between August and December. We observed males in vocalization at the beginning of the rainy season like whistling, and then breeding took place in small burrows in the edges of ponds, with eggs and tadpoles in developing. It is in the IUCN Red List Least Concern category, not threatened. Due to tolerance to degradation of their environment, their populations are stable at present. It occurs throughout Brazil, in the

Atlantic Forest, Cerrado and Pantanal, being found in open fields, pastures, swampy areas, degraded forests and urban habitats [46].



Figure 5. A. *Trachycephalus typhonius*. B. *Leptodactylus fuscus*.

- (2) *Leptodactylus labyrinthicus* (Figure 6A) presented peaks of vocalization between July and December, with young and adults walking around the pond area, where it constructed foam nests with many eggs and tadpoles enters the more closed vegetation. We observed youngsters moving around on the floor in the months before the reproductive period. It is in the IUCN Red List Least Concern category, not threatened. It is an adaptable species to anthropogenic regions and presents stable populations. It is distributed over open areas in the Midwest, South, and Southeast of Brazil. We can see this species in the Atlantic Forest, Cerrado, Caatinga, in plateaus and edges of the Pantanal, at altitudes of up to 1,000 m [47].
- (3) *Leptodactylus latrans* (Figure 6B) was observed for almost the entire year, on the banks of the pond. In the dry periods, between April and August, the presence of young prevailed. In early September, we observed adults taking care of their tadpoles inside the pond, swimming together with the offspring. The adults showed defensive behaviors of their offspring, with leaps towards the observers, with acute and brief vocalization. It is in the IUCN Red List Least Concern category, not threatened. It easily adapts to changes in its habitat, being found in rural areas, secondary habitats, and urban areas. Hunting for food and fires can impact local populations. It is widely distributed in the states of Amazonas, Bahia, Ceará, Rio de Janeiro, Espírito Santo, Minas Gerais, Paraíba, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina and São Paulo, in the Atlantic Forest, Cerrado and Pantanal, occurring up to 1,400 m altitude [48].



Figure 6. A. *Leptodactylus labyrinthicus*. B. *Leptodactylus latrans*.

- (4) *Leptodactylus mystacinus* (Figure 7A) was observed only in March. It reproduces in the rainy season constructing foam nests in underground chambers, where it lays its eggs. Its reproductive activity in southeastern Brazil was reported for October, although the species can potentially reproduce in August, September, and November, as cited in reference [49]. It is in the IUCN Red List Least Concern category. The species adapts well to anthropogenic disturbances, is not threatened, and its populations are currently stable. It is distributed throughout the East and Midwest of Brazil, in the Atlantic Forest, Cerrado and Pantanal, being observed on the ground near water bodies, in deforested areas, in riparian forest subject to flooding and pasture fields, up to 1,800 m altitude [50].
- (5) *Leptodactylus podicipinus* (Figure 7B) showed nocturnal reproductive activity on the ground among the vegetation on the banks of the pond placing its eggs in foam nests. It presented peaks of predominant vocalizations in February, with an interruption in the drier months, starting to appear again in September with the new rains. It is in the IUCN Red List Least Concern category. It is not currently threatened because of its wide tolerance to degradation of its habitat, being distributed in stable populations. It has a central and western distribution in the Brazilian territory, being observed in the Cerrado formations, which extend along with the Rio Madeira and Rio Amazonas, and in the Pantanal, being able to occur in up to 1,000 m of altitude [51].



Figure 7. A. *Leptodactylus mystacinus*. B. *Leptodactylus podicipinus*.

- (6) *Physalaemus cuvieri* (Figure 8A) was observed it vocalizing on the banks of the pond in small depressions, where they built their foam nests with eggs, connected to the grass stems at the water's edge. Its vocalization peaks were in the rainy season in November and December. It is in the IUCN Red List Least Concern category, not threatened. It is a very adaptable species to the anthropic environment, presenting stable populations. It distributes through the northeast, central, southeast and south regions of Brazil, in the Atlantic Forest, Cerrado, and Pantanal, at up to 2,000 m of altitude [52].
- (7) *Physalaemus nattereri* (Figure 8B) was observed it in explosive reproduction using temporary puddles formed by the October rains. It was observed inside nests of

communal foam containing their eggs on the margins of these temporary puddles. Inside these nests, we observed too *T. typhonius* and *Dermatonotu muelleri* found during the day, probably serving as a shelter for these species. The foam nests broke up in around two days, data corroborated data from reference [53]. The presence of *P. nattereri* in the study area was reinforced by its fossorial and seasonal, and well adapted to the semi-arid continental climate, in a region that was constituted in the past by a percentage of a kind of savannah environment [54]. It is in the IUCN Red List Least Concern category, but *L. nattereri* does not adapt well to anthropogenic disturbances by the expansion of intensive agriculture in the Cerrado biome, which has affected its local populations, which are in decline. It occurs in the Midwest, Northeast, and Southeast of Brazil, in the Cerrado and Pantanal, being found at up to 1,500 m of altitude. It is fossorial and seasonal, well adapted to the semi-arid continental climate [55].

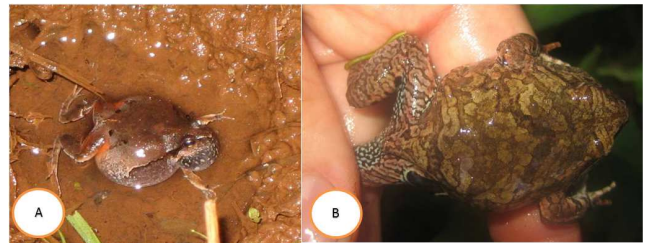


Figure 8. A. *Physalaemus cuvieri*. B. *Physalaemus nattereri*.

3.3. Family Bufonidae

The family Bufonidae was represented by two species in the 2009 inventory: (1) *Rhinella diptycha* [56]; (2) *Rhinella mirandaribeiroi* [57]. One species was registered in the 2010 inventory: *R. diptycha*.

- (1) *Rhinella diptycha* (Figure 9A) was observed in reproductive activity on the banks of the pond or in the water, with its peak of vocalization between July and August, despite being seen until December. In the 2010 inventory, we observed *R. diptycha* in explosive reproduction during the rainy season from October to December, placing a high amount of eggs also in the puddles formed by the rains. It is in the IUCN Red List Data Deficient category. It is a species adapted to anthropogenic disturbance, commonly seen in urban areas. Their population status is not very well known, with little information. It distributes throughout the Midwest, Northeast, and Southeast regions of Brazil, from Ceará to the Rio Grande do Sul, at up to 2,000 m of altitude. It lives in several habitats, including the Chaco, Cerrado, and Atlantic Forest regions [58].
- (2) *Rhinella mirandaribeiroi* (Figure 9B) showed explosive reproduction, with peak vocalization between August and September. We observed males actively vocalizing over pieces of floating trunk in the shallowest part of the pond. It is in the IUCN Red List Least Concern category. The species is not currently threatened, with stable

populations. It is distributed mainly through the Southeast and Northeast regions of Brazil, in the Atlantic Forest, Caatinga, and Pantanal and open formations along with the Madeira, Amazon, Tapajós, and Xingu rivers, reaching an altitude of 1,500 m [59].

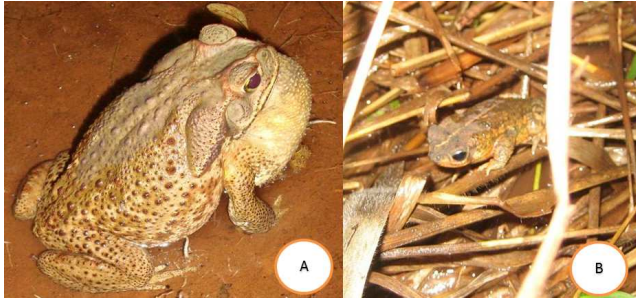


Figure 9. A. *Rhinella diptycha*. B. *Rhinella mirandaribeiroi*.

3.4. Family Microhylidae

The family Microhylidae was represented by just one species in the 2009 inventory: (1) *Elachistocleis* cf. *cesarii* [60]. Three species was registered in the 2010 inventory: (1) *Elachistocleis* cf. *cesarii*; (2) *Chiasmocleis albopunctata* [61]; (3) *Dermatonotus muelleri* [61].

(1) *Elachistocleis* cf. *cesarii* (Figure 10A) was found associated with termite mounds near the lake in March and April 2009. The species has terrestrial and fossorial habits, being found in open areas near lakes, in litter and tree holes in tropical forests, in Bromeliads and over the forest edge, and pastures [62]. It showed its peaks of vocalization in September 2009. We also observed it vocalizing in puddles formed by the rains between October and December in 2010 inventory. There is no information about it in the IUCN Red List. *Elachistocleis bicolor* is tolerant to some modification of its habitat even local populations are at risk by factors associated with agriculture, logging, fire, infrastructure development, and water pollution [63]. Maybe we could use this information to know more about *E. cesarii*. It is distributed throughout the Brazilian territory, in the Atlantic Forest, Cerrado, and Pantanal, at up to 500 m of altitude [64].

(2) *Chiasmocleis albopunctata* (Figure 10B) was observed vocalizing and reproducing in temporary puddles formed during rainy periods in 2010. It is in the IUCN Red List Least Concern category, not threatened, despite being harmed by fires and by the spread of intensive agriculture. The species adapts easily to anthropogenic disturbances and is usually associated with temporary water bodies and wetlands in forests, pastures, and fields. The species has been located in different regions of the state of São Paulo, Bahia, Maranhão, and Tocantins [65] and according to reference [66], can be considered the species with the greatest altitudinal and latitudinal variation, showing the greatest environmental plasticity. It is distributed throughout the Federal District and the states of Goiás,

Mato Grosso, Mato Grosso do Sul, Minas Gerais, and São Paulo, in open areas in the Chaco and Cerrado, living between 100 and 1,500 m of altitude [67].

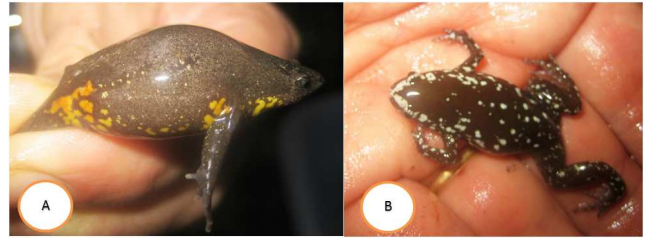


Figure 10. A. *Elachistocleis* cf. *cesarii*. B. *Chiasmocleis albopunctata*.

(3) *Dermatonotus muelleri* (Figure 11) showed explosive reproduction in temperate pools formed by rains, between October and December. We observed it digging into the soil at the edges of puddles that were drying in the intense heat. Studies in reference [68] showed that *D. muelleri* had a high degree of specialization in arid environments, resulting from the pronounced dry season faced by the species, a factor that strongly acts in the selection of the excavation tactics with the construction of underground chambers. It is in the IUCN Red List Least Concern category. Although the species does not adapt so well to anthropogenic disturbances, its populations are currently stable, not being threatened. It is distributed through the Midwest, Northeast, and Southeast of Brazil, in the Cerrado and Pantanal, being found in up to 1,500 m of altitude [69].



Figure 11. *Dermatonotus muelleri*.

3.5. Analyzing the Study Area for the Years 2009 and 2010

The majority of species inventoried is well known distributed to Brazilian territory and their presence in the study area was no surprise. In different ways, the registration of *P. bolbodactyla* seems to be an essential finding since no study has mentioned the presence of this species in the São Paulo state. Another important finding was the reporting of *R. mirandaribeiroi* that its first registration for São Paulo state, was recently (in 2015) made in the municipality of São Joaquim da Barra, located 30 km from our study site [70].

Identifying this species in the study area was the second identification for the state of São Paulo for this species. At first glance, it seems contradictory to the remarkable species richness inventoried in 2009 for this small ecological impacted area in the middle of a soy monoculture. Let's try to understand it. The anuran species that inhabited the study area were generalist and well adapted to anthropic environments, living more than one biome in Brazil. The reference [71] showed that breeding sites, like artificial agricultural ponds located at the edge and close to forest fragments, supported greater abundance and species richness of anurans. They estimated how closer the breeding sites are to forest fragments more richness the anurofauna will be. The forest fragments are

essential for shelter and foraging needs. We located a forest corridor with 1,700m of extension, distant 450m from the study site (Figure 12). The forest corridors that link isolated forest fragments are unequivocally essential for amphibian's survive [72]. This ecological corridor in the study area is flanked by agricultural areas is connected to a 10.0 ha forest fragment. It is separated from another forest fragment (18.5 ha) by a train line. This last forest fragment is separated too by another forest fragment (3.3 ha) by a state highway (SP 330). You can see this information in Figure 12. Note the presence of a eucalyptus plantation with 13 ha and a municipality dam with a surface area of 19 ha. We didn't get any information about these two ecosystems near to study area.



Figure 12. Google Earth image from February 2021 took at 688 m of height. 1. Study area with 3.0 ha; 2. Ecological forest corridor with 1,700m of extension. It is 450 m distant from the study area; 3a. Forest fragment with 10.0 ha; 3b. Forest fragment with 18.5 ha; 3c. Forest fragment with 3.3 ha; 4. Agricultural areas; 5. Train line crossing two forest fragments; 6. State highway crossing two forest fragments; 7. Municipality dam with 19 ha of surface area.; 8. Eucalyptus plantation with 13 ha.

We question whether these species are remnants of forest fragments that previously existed there or could then be able to traverse it and risk themselves to cross open habitats? Despite the low dispersal capacity of amphibians cited by their skin physiology and thus having a strict dependence on moisture [73], some species move distances greater than 10 km [74]. The reference [75] observed in Brazilian Atlantic Forest that generalist species of anurans usually move between pasture and the forest habitat despite its inherent risks when there are water sources. The reference [71] highlights the importance of the proximity and size of forest fragments to agricultural areas. These remaining forest fragments could provide refuge from the heat and protect from water stress which is probably a problem of open agricultural areas for anurans. The reference [76] showed that the richness of anuran species in ponds in agricultural areas decreases with high

densities of human residents and conversely increases substantially when forest fragments are surrounded. Our studies reinforce the importance that the pond in study area had as a breeding site for the local anurofauna. All inventoried species showed to be performing their reproductive cycles with vocalization, courtship, competition for mates, and egg deposition. Factors such as water conductivity, presence of vegetation and fish, shading, and proximity to urban buildings define the integrity of a pond's amphibian community [76]. The high conductivity of water, which may be related to the presence of pollutants, ions, and nutrients, has a negative effect on the development and maintenance of this community [77]. Unfortunately, we did not measure the conductivity of the pond in our study area. Despite this, we have rarely seen animals with malformations probably resulting from mutagenic factors. The pond had a low density of fish, a

positive factor because they were predators. The abundant vegetation in the pond was a positive factor. It could reduce tadpoles' predation that uses the aquatic plants to hide [78]. The lacked shading was another positive factor resulting in higher water temperatures [79] contributing to larval growth and better feeding rates. Usually, the shading on ponds results from urban buildings. Another positive factor for this study pond was the existence of a few homes in its vicinity. But what would be the main factor that led to the pond's complete depletion in 2010? This pond appeared to be formed from the damming of a natural water flow that fed it from a large pipe. Our investigation led us to conclude that this stream that fed it was cut off. This factor added to dry periods of the year, causing the lake to dry out in the second half of 2010. This event, unfortunately, eliminated the entire anuran community established in the pond. When the rains started in October 2010, they were not enough to recover the initial volume of water. In 2010 we could observe just species of explosive reproduction using the place with the puddles formed by the heavy rains (Figure 13). The rain is the mean environmental factor linked with explosive breeding events. The reference [80] showed that explosive breeding species respond to rain consistency during the 48 to 72 hours and the amount of rain in 24 hours. Unquestionably, we watched here an aggravating environmental deterioration.



Figure 13. A. Ephemeral ponds formed after storm rains in late spring and beginning of summer. B. Eggs deposited in aquatic vegetation and the pool.

3.6. Analyzing the Study Area for the Year 2021

Twelve years after the first inventory, the study area showed a high level of degradation. The study area was transformed into a pasture with grasses for cattle confinement and no longer has any body of water that can shelter the anurofauna. No temporary puddles, at least with tadpoles, were observed even in periods of torrential rain. The soil is compacted possibly by the trampling of cattle and has large gullies (Figure 14). Unfortunately, we can see a high rate of environmental degradation with the loss of biodiversity of anurans that previously inhabited the study area.



Figure 14. A. Panoramic view of the study area in 2021. Note that this was the place occupied by the pond in 2009; B. Vegetation in 2021 formed mainly by grass bushes in the central region occupied by the pond in 2009; C. A gully in the old pond margins observed in 2021; D. Cattle feces observed in 2021 in the pasture formed in the place occupied by the pond in 2009.

4. Conclusion

From this study we can draw the following conclusions:

- 1) Until the year 2009, there was a community composed of an anurofauna living in the study area. The species could reproduce, obtained conditions for its subsistence, and showed themselves to be opportunistic and tolerant to anthropic areas, like this agricultural area, in the municipality of Ituverava, São Paulo State, Brazil.
- 2) This anuran community surprised us by showing relative richness, including by presenting species not well known for the State of São Paulo, such as *Pseudis bolbodactyla* and *Rhinella mirandaribeiroi*.
- 3) In 2010, after the pond had dried up, this community was drastically affected, with only a few species of explosive reproduction being observed.
- 4) In 2021, the study area showed a high level of ecological deterioration, with no kind of body water, with no evidence of an anurofauna associated with it.

This study highlights the worrying reality of environmental degradation and its relationship with anurofauna in Brazil. Agriculture's expansion is impacting many populations of amphibians, even before the details of their species are known. Even species adapted to areas ecologically degraded by human action need to be studied for their protection. What are and how do they face environmental stresses? These questions await further study.

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