

## Research Article

# Forest Fragments Surrounded by Sugar Cane Are More Inhospitable to Terrestrial Amphibian Abundance Than Fragments Surrounded by Pasture

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In recent years, there has been increasing interest in matrix-type influence on forest fragments. Terrestrial amphibians are good bioindicators for this kind of research because of low vagility and high philopatry. This study compared richness, abundance, and species composition of terrestrial amphibians through pitfall traps in two sets of semideciduous seasonal forest fragments in southeastern Brazil, according to the predominant surrounding matrix (sugar cane and pasture). There were no differences in richness, but fragments surrounded by sugar cane had the lowest abundance of amphibians, whereas fragments surrounded by pastures had greater abundance. The most abundant species, *Rhinella ornata*, showed no biometric differences between fragment groups but like many other amphibians sampled showed very low numbers of individuals in fragments dominated by sugar cane fields. Our data indicate that the sugar cane matrix negatively influences the community of amphibians present in fragments surrounded by this type of land use.

## 1. Introduction

Species persistence in fragmented landscapes may heavily depend on their tolerance to the surrounding-environment matrix [1–3]. The surrounding matrix can influence resource availability [4], animal dispersion [5], habitat fragment occupation [2, 6], and also the distribution and population dynamics within the fragment [1, 7, 8]. Generally, the higher the structural similarity of the matrix with the fragment, the greater the gene flow and dispersion of animals [1], as well as the richness and abundance of mammals [9], birds [10], and amphibians [11].

The matrix importance to the response of species to fragmentation depends not only on its structural characteristics, but also species biology [12]. Amphibians are sensitive

to environmental alterations, because most have a biphasic life cycle [13], permeable skin [14], low vagility [15], and strong philopatry [16]. Also, they have been suffering declines worldwide, mainly due to habitat loss, overutilization, and chytridiomycosis which is an infectious disease caused by fungus [17, 18].

In surveys focused on amphibians and matrices, there is predominance of studies with pastures. Deforestation for pasture establishment can lead to richness reduction and the predominance of generalist and terrestrial anurans [19]. The pasture matrix can also reduce reproductive success of *Phyllomedusa tarsius* [20], amphibian richness [21], and abundance [22]. Pastures negatively impact amphibians, especially large and terrestrial species whose eggs are deposited on land, but whose larvae develop in water [23].

Our goal was to compare terrestrial amphibian diversity and biometrics of the most abundant species between forest fragments under the influence of two predominant matrix types (sugar cane and pastures). Our premises are that amphibian diversity, the weight and length of the most abundant species, will be greater in the fragments surrounded by sugar cane plantations and the opposite in fragments with pastures. We assume this because a larger number of studies indicate negative effects of grazing on frogs and due to the scarcity of studies on sugar cane.

## 2. Methods

**2.1. Study Site.** We conducted the study in three cities—Alfenas, Areado and Serrania—in the state of Minas Gerais, southeastern Brazil, transitions between biomes of the Atlantic Forest, and Cerrado. The region has an average altitude of 880 m, an average annual temperature of 23°C, average annual rainfall of 1600 mm, distributed evenly throughout the year, and an annual average 70% relative humidity [24, 25]. We collected the data in fragments of semideciduous seasonal forest whose landscapes have been highly fragmented and altered by agricultural activities, with only 3% of native forest in different successional stages [26]. The matrix surrounding the fragments was quite diverse, but predominantly sugar cane and pasture crops were cultivated in them [27, 28] (Figure 1).

**2.2. Sampling Design.** In the study area, we selected six semideciduous seasonal forest fragments, according to the following five criteria: (1) minimum distance of 500 m between fragments to ensure independence between samples [14], (2) area between 15 and 100 ha, (3) shape index [29] between 1.22 and 2.46, (4) presence of a water body in or around the fragment, and (5) one of the two predominant types of matrices (over 75% of the surroundings); that is, in the chosen fragments, sugar cane or pastures prevailed. Thus, we selected three fragments with each of these predominant matrices (Figure 1).

In each of the six fragments, we installed five pitfall traps with drift fences. Each trap array consisted of four 30 L buckets buried in the ground up to the top of the bucket, with a central one and three radial buckets four meters away from the central bucket and placed at 120° from each other. These radial buckets were connected to the edge of the central bucket by plastic fences 0.5 m tall and 4 m long. Traps were 50 m from the edge of the fragment and 30 m apart from each other. We collected the samples over a period of 15 consecutive days in January 2011 and six nonconsecutive days in December 2011, totaling 22,680 trap-hours.

We collected each specimen under IBAMA License no. 1934-1 and we measured with a caliper (accuracy 0.01 mm), weighed with a precision balance (0.1 g), euthanized in saturated cloretone and ethanol in water [30], fixed in 10% formalin and preserve in 70% alcohol, according to ethical and legal principles [31, 32]. We preserved the collected material in the Herpetological Collection Alfred Russel Wallace (CHARW) of the Federal University of Alfenas (Unifal-MG). Subsequently, we determined the sex of the individuals collected by dissection and direct observation of the gonads.

**2.3. Statistical Analyses.** We compared the terrestrial amphibian diversity (richness and abundance) between fragments with each of the two predominant matrix types (sugar cane and pasture) by species rarefaction curves [33] with EstimateS 7.5.0 [34] and 500 randomizations. The program generated 500 species accumulation curves, randomizing the order of the samples. Thus, each point on the curve corresponds to the richness average accumulated in the 500 associated curves with a standard deviation. Several studies support this analysis method rather than the use of classical diversity indices [21, 23, 33, 35, 36]. We compared the observed richness with estimated richness by three nonparametric estimators of EstimateS 7.5.0: Chao 1, Chao 2, and Bootstrap, recommended by different authors [36, 37] and used in similar studies with amphibians [21, 23, 36]. We relate the observed and estimated richness through the efficiency measure [36]. We compared the species composition of fragments with different matrix types through the concept of complementarity [23] and significance level according to Silva et al. [19]: values above 50% indicate high beta diversity. We did not consider exotic species in these analyses.

For biometric analysis, we measured data from snout-vent length (SVL), biomass, and mean biomass/SVL, from individuals of the most abundant species (*Rhinella ornata*), which we collected in January 2011. This analysis is a more subtle and refined scale for determining the matrix influence on the forest fragment biota [38–40]. After all, before declining and disappearing, a population under impact should show signs of withering, such as reduced average growth or weight loss. The Shapiro-Wilk test rejected the sample normality ( $P < 0.01$  for all samples), so we compared the measurements of males and females by Mann-Whitney test for each matrix type. We did not find sexual dimorphism, so we pooled sexes for a comparison between the two matrix types by Mann-Whitney. We did not test the sexual dimorphism for the fragments with a predominance of sugar cane because of the low number of males in the sample ( $N = 2$ ).

## 3. Results

In total, 190 individuals were captured from seven species belonging to five families (Table 1). The most abundant species was *Rhinella ornata* with 121 specimens (63.7% of total individuals). Two species (*Odontophrynus cultripes* and *Leptodactylus mystacinus*) had only one or two specimens collected. Of the total number of collected individuals, 87.9% ( $N = 167$ ) were recorded in predominant pasture fragments and only 12.1% ( $N = 23$ ) in sugar cane. One recorded species, *Lithobates catesbeianus*, is non-endemic and, although listed in Table 1, was excluded from further analysis. Excluding this anuran, which is especially abundant and was introduced recently in a single pasture fragment, the difference between amphibian total abundance in fragments with predominant sugar cane and pastures ranged from 101 to 144. This species alone accounted for 25.7% of individuals captured in pasture fragments.

Analysis of observed and estimated richness showed relatively little difference between them for sugar cane and

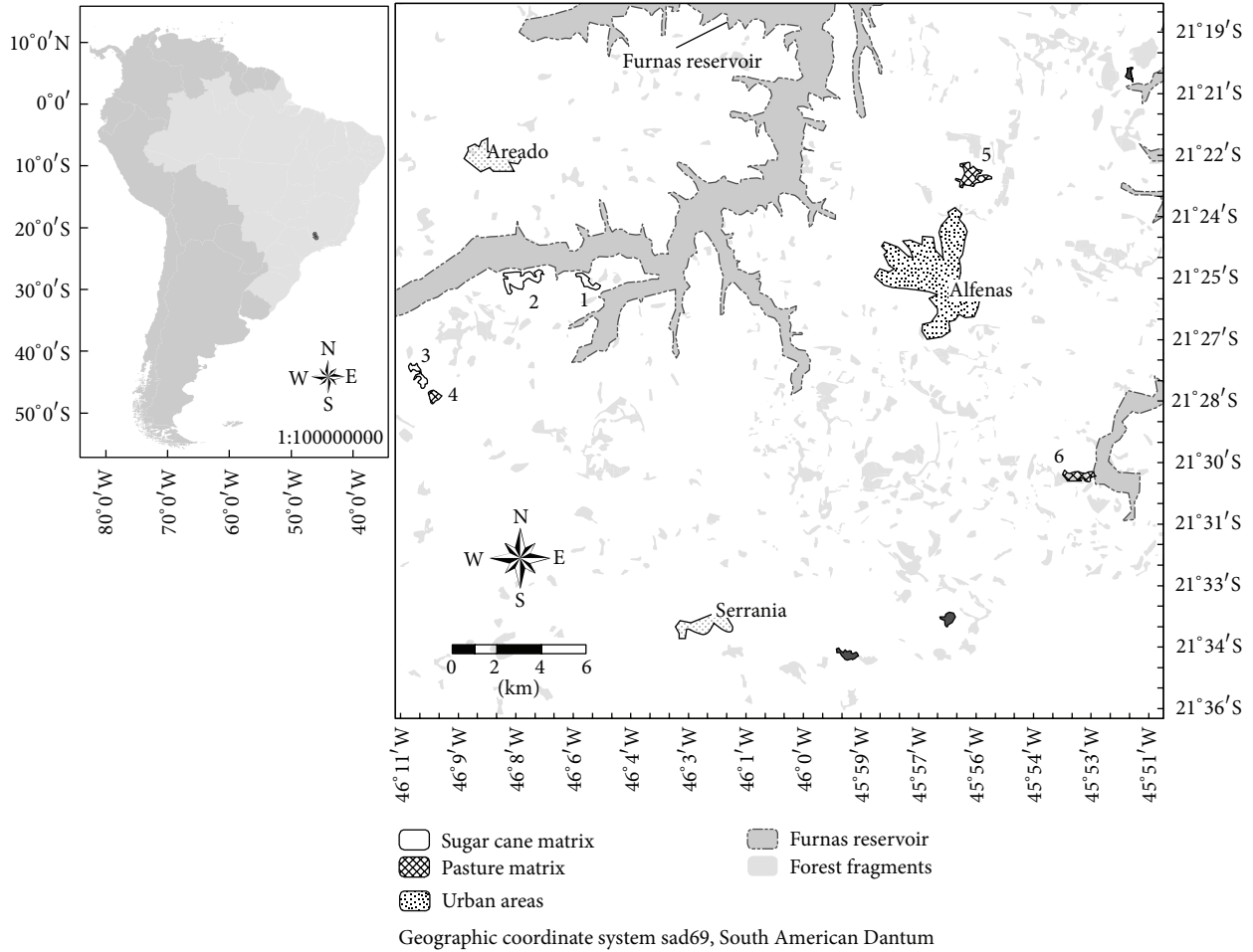


FIGURE 1: Study area location in the surroundings of the cities of urban areas, in the state of Minas Gerais, Brazil, and the six forest fragments sampled.

TABLE 1: List of terrestrial amphibian species and their abundance in fragments of Semi-deciduous seasonal forest with two predominant matrix types (sugar cane and pastures).

Species	Matrix		Total
	Sugar cane	Pasture	
Bufonidae			
<i>Rhinella ornata</i>	15	106	<b>121</b>
Craugastoridae			
<i>Haddadus binotatus</i>	4	7	<b>11</b>
Cycloramphidae			
<i>Odontophrynus cultripes</i>	1	0	<b>1</b>
<i>Proceratophrys boiei</i>	0	8	<b>8</b>
Leptodactylidae			
<i>Leptodactylus mystacinus</i>	2	0	<b>2</b>
<i>Physalaemus cuvieri</i>	1	3	<b>4</b>
Ranidae			
<i>Lithobates catesbeianus</i>	0	43	<b>43</b>
<b>Total</b>	<b>23</b>	<b>167</b>	<b>190</b>

TABLE 2: Observed species richness ( $S_{obs}$ ), estimated species richness ( $S_{est}$ ) for three estimators and efficiency ( $S_{obs}/S_{est}$  average) of terrestrial amphibians in the fragments of Semi-deciduous seasonal forest with two predominant matrix types (sugar cane and pastures).

Matrix	$S_{obs}$	$S_{est}$			Efficiency
		Chao 1	Chao 2	Bootstrap	
Sugar cane	5	5.5	5.32	6	0.89
Pasture	4	4	4	4.25	0.97

pasture, with efficiencies greater than 85% (Table 2). This result indicates that the sampling effort was sufficient.

The complementarity value was 50%. In other words, half of the species of the sugar cane fragment list differed from the list of the fragments with pastures. On average, matrices of sugar cane and pastures showed little permeable traffic for half of amphibian species observed.

Like the comparison of observed and estimated richness, sample-based rarefaction curves (Figure 2) indicate that the sampling effort was sufficient, with the two matrix types tending to stabilize. Individual-based rarefaction curves

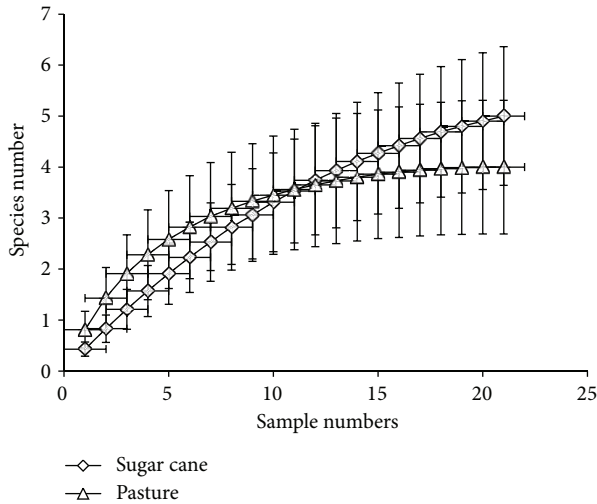


FIGURE 2: Sample-based rarefaction curves of terrestrial amphibians in fragments of Semideciduous Seasonal Forest with three predominant matrix types (sugar cane and pasture), estimated from 500 randomizations in the order of the samples.

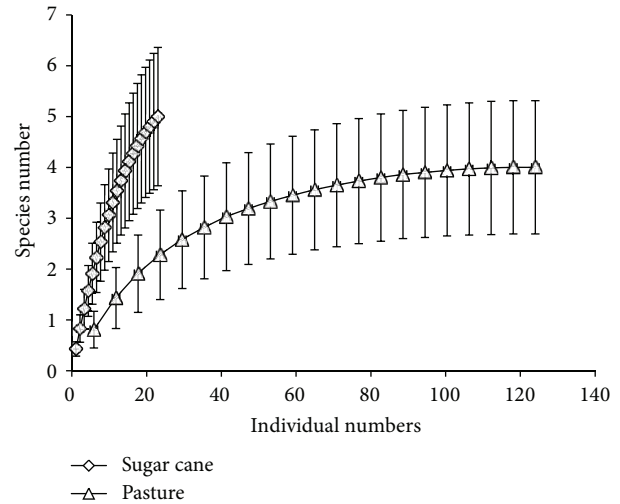


FIGURE 3: Individual-based rarefaction curves of terrestrial amphibians in fragments of Semideciduous Seasonal Forest with three predominant matrix types (sugar cane and pasture), estimated from 500 randomizations in the order of the samples.

(Figure 3) support the results of Table 1: richness relatively is constant, but relative abundance is very different. Sugar cane fragments exhibited the lowest individual number.

*Rhinella ornata* biometric analysis detected no sexual dimorphism in relation to biomass (pasture:  $U = 459.5$ ,  $Z(U) = 1.099$ ,  $P = 0.498$ ), snout-vent length (SVL) (pasture:  $U = 447.5$ ,  $Z(U) = 0.817$ ,  $P = 0.414$ ), nor to ratio biomass/SVL (pasture:  $U = 462.5$ ,  $Z(U) = 0.642$ ,  $P = 0.521$ ). Also no biometric differences were observed between fragments with the two predominant matrix types (biomass:  $U = 461.5$ ,  $Z(U) = 0.47$ ,  $P = 0.64$ ; SVL:  $U = 477$ ,  $Z(U) = 0.29$ ,  $P = 0.76$ ; biomass/SVL:  $U = 450$ ,  $Z(U) = 0.59$ ,  $P = 0.55$ ).

#### 4. Discussion

Forest fragments surrounded by two predominant matrix types (sugar cane and pastures) showed constant values of terrestrial amphibian richness, but the total abundance varied greatly. The lowest individual number was present in fragments surrounded by sugar cane, about seven times less than that in fragments with pasture. Some studies indicate that abundance is a more efficient bioindicator parameter than richness [22, 41]. The search for species richness differences may mask other processes and generate inefficient conservation plans [19]. One reason for this may be a generalist species presence occupying disturbed areas, which can artificially inflate species richness [42, 43].

Fragments surrounded by sugar cane with low abundance may be the result of management practices used in this cultivation and not in the other two activities. Although the pastures are more like open habitats, they can be considered relatively perennial. Sugar cane management, in turn, requires cutting stalks every year or one and a half years, producing instant and drastic changes in the landscape.

Suddenly after cutting, amphibians of the fragment, which explore the sugar cane plantation in search of resources or simply use it as a transit corridor to reach breeding areas, are more exposed to predators [44], high UV-B radiation [45], high temperatures, and low air humidity [46].

Another management practice used in sugar cane is fire. Several negative impacts from slash-and-burn practices have been reported for amphibians and other organisms [47, 48], but this method is still used in certain agricultural activities. Cattle ranchers sometimes use this technique to clear forest or brush area and thus facilitate establishing pasture. However, afterwards, controlled burning is not used. Currently in sugar cane fields, the practice of burning straw to facilitate harvesting is widely used throughout the year [49]. Individual amphibian deaths from such fires appear to be rare events [50, 51], because it is easy for them to find refuge [52]. However modification or destruction of habitats used for foraging, shelter, and reproduction is very common. In semideciduous seasonal forests, fire has a deleterious effect on anuran litter richness and abundance. After the fire, the leaves, which again cover the ground, are compressed litter that does not retain moisture, making amphibian recolonization difficult [39]. Another fire hazard is the possibility of reaching vegetation close to the sugar cane fields and eliminating natural predators of some pests, requiring greater herbicide and pesticide use to control weeds that grow rapidly after burning [53].

Sugar cane is one of the main consumers of herbicides and pesticides in Brazil [54]. Pastures, in turn, employ no pesticides. The herbicide, Tebuthiuron, used exclusively in sugar cane farming, can cause weight loss [55], relating directly to amphibian health or adaptability [56]. Another widely used herbicide in sugar cane is Diuron, which causes deformities in embryos and tadpoles, affecting amphibian



growth and survival [57]. The amount and type of pesticides used around fragments may explain the terrestrial amphibians low abundance associated with sugar cane compared to fragments surrounded by pastures.

The expected negative effects of pasture [19], contradicted by our results, may be simply due to the larger number of studies about amphibians and pastures [19, 21, 22, 43, 58–63] than in other matrix types [64–67]. Studies on sugar cane effects in amphibians are particularly scarce. This matrix, when studied, was never exclusively or quantitatively analyzed and always embedded in a matrix being predominantly covered in pasture [19, 60, 63, 67–69]. Still, some studies have shown that temporary ponds or lagoons in pasture areas (often used as a cattle water source) are an important resource as breeding sites for several amphibians species and may be an important management strategy for conservation purposes. However, the recent sugar cane boom has reduced this possibility [63, 64]. At least for studies in Brazil, the large number of pasture studies may reflect this matrix being predominant compared to agricultural crops: 9% of the country is occupied by sugar cane fields, while pastures cover over 20% of the territory [70].

Another aspect that should be considered when comparing fragments surrounded by different predominant matrices is species composition, which enables distinguishing matrix effects on different species groups, such as, for instance, generalist, exotic, and specialist. Many of the species observed in the sampled fragments are generalists and theoretically less dependent on forest environments. It can be argued that the occurrence of many of these species is favored by deforestation and the formation of open areas resulting from land use activities [40]. This is undoubtedly the case for *Physalaemus cuvieri* [43, 65, 68, 71–74]. Other species are not so exclusively characteristic of open areas but have generalist habits, being found both in disturbed habitats, as well as in forests, as *Odontophrynus cultripes* [72, 74, 75] and *Leptodactylus mystacinus* [65, 69, 73, 76].

It is important to note, however, two aspects of the considered generalist species: (1) areas with marked climatic seasonality (as many semideciduous forests) may present natural predominance of these species, not only for the forest land conversion into cultivated areas [43] and (2) even for such species, forest fragments are fundamental as refuge areas during the dry season, shelter during the reproductive period or as a source of food resources [60]. Although they are generalist species, these species may also be affected by certain matrix types. Of all the species considered generalists in our study, two (*O. cultripes* and *L. mystacinus*) were recorded in low numbers, which does not allow generalizations, but two *P. cuvieri* confirmed the trend of low abundance in fragments with predominance of sugar cane.

Exotic species many times also show generalist behavior. The bullfrog, *Lithobates catesbeianus*, fits this situation. It was present in the study area, only in fragment 5, with pasture being the predominant matrix. It was the third most abundant species in this study, with 43 specimens found. This frog was introduced in several locations in Brazil and the world, from frog farms for meat production. This species has had negative consequences for native assemblages [71, 77].

During our study, this species was released by the owner at a lake adjacent to the fragment to serve as a food source. It was possible to observe the individual recruitment and population growth at the site. Compared to other records of the species in Brazil [77], this is the first record in the Alfenas region.

Species composition analyses and their ecological requirements are also important for positive biomarker identification. In this case, it would be a more demanding species, associated with forest environments and theoretically more sensitive to the fragmentation effects and less permeable matrices, such as *Haddadus binotatus* [71, 73, 74], *Proceratophrys boiei* [65, 71, 74, 78], and *Rhinella ornata* [69, 71, 73, 76, 78]. These three species were present in low abundance or absent, case of *P. boiei*, in fragments surrounded by sugar cane contributing to the conclusion that a sugar cane matrix is inhospitable to the more demanding species.

The number of individuals of *H. binotatus* recorded in fragments with pasture is almost double the abundance in fragments with sugar cane. Nevertheless, the total abundance of this species was underestimated because individuals can escape pitfall traps by jumping or climbing out of the bucket [71, 79]. But, considering that this ability to escape is species intrinsic and it is not affected by the matrix surrounding the fragments, their relative abundance can be compared. *Proceratophrys boiei* is the second most abundant species and absent in sugar cane fragments. One possibility is that this species has suffered a decline to the point of local extinction in these fragments. *Rhinella ornata* is the first in abundance as in [71] and, although present in fragments surrounded by sugar cane, the number of individuals in fragments with this matrix type was minimal. However, the expectation of finding biometric differences between samples, particularly with smaller individuals in fragments surrounded by sugar cane, was not supported. Maybe this kind of difference, because it is more subtle, requires more refined data and analysis, for instance, geometric morphometric, which considers not only the size but also the shape and the symmetry of the body [80].

Another hypothesis is that *R. ornata* is not a forest bioindicator or forest species as previously thought. Some studies confirm this species as a forest species and being associated with the Atlantic Rainforest biome *lato sensu*, however with great ecological plasticity and high dispersal capacity, also occupying disturbed habitats and urban areas [75, 81]. Species capable of using the matrix or at least those species that tolerate matrix effects, normally maintain stable population or may even grow [1]. Although *R. ornata* can provide large displacement capacity and dominate numerically varied environments, we do not believe that this species is immune to any kind of matrix. This species has been losing habitats in the state of São Paulo due to sugar cane expansion [69], and some isolated population has no gene flow between them [82]. The low abundance observed in fragments surrounded by sugar cane in the present study confirms the possibility that this species and other terrestrial amphibians are negatively affected by the advance of sugar cane.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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