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Comparing leaf litter anuran diversity in two habitats of an Atlantic Forest area in Rio de Janeiro State, Southeastern Brazil

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ABSTRACT

We compared species richness, relative abundance, density (ind/100 m^2) and biomass per hectare (g/ha) of leaf-litter anurans between forest and restinga habitats in an insular Atlantic Forest area in southeastern Brazil. The local assemblage of leaf litter anurans was composed by nine species (eight of them occurring in the forest and six in the restinga), of which the most abundant were *Ischnocnema parva* and *Adenomera marmorata* in the forest, and *I. parva* and *Dendrophryniscus lauroi* in the restinga. The estimated local density of leaf-litter anurans was 11.7 ind/100m^2 (biomass per hectare = 848.4 g/ha) in forest and 7.3 ind/100m^2 in restinga (biomass per hectare = 262.8 g/ha). Anuran species richness, estimated densities and biomass in the restinga were lower than that of the forest, probably due to the less favorable environmental conditions of restingas for amphibians.

Key Words: Amphibian survey; Frog; Ilha Grande island; Restinga; Tropical forest.

The Brazilian Atlantic Forest is considered one of the world's 36 biodiversity hotspots, having irreplaceable threatened areas with very high species richness and rates of endemism (Mittermeier *et al.*, 2011; CEPF, 2016). This biome is important with respect to amphibian diversity and conservation (Hrdina and Romportl, 2017) because approximately 625 amphibian species are known to occur within its limits, and most of them are endemic (Rossa-Feres *et al.*, 2017). Nevertheless, new species are still being steadily described from this biome (e.g., Cruz *et al.*, 2019; Silva *et al.*, 2020; Nunes *et al.*, 2021).

While assemblages of leaf litter frogs have been relatively well-studied in forests within the Atlantic Forest domain (e.g., Giaretta *et al.*, 1999; Dixo and Martins, 2008; Siqueira *et al.*, 2009; Santos-Pereira *et al.*, 2011; Vagmaker *et al.*, 2020), they have been less studied in other ecosystems within this biome, such as the restingas, which are open coastal habitats formed by sand dunes covered by herbaceous, shrubby and low arboreal vegetation (Suguio and Tessler, 1984; Araújo, 1992). Nevertheless, studies in restinga areas have increased in recent years, expanding the knowledge about amphibian assemblages from this ecosystem (e.g., Oliveira & Rocha, 2015; Gondim-Silva *et al.*, 2016; Mageski *et al.*, 2017; Oliveira *et al.*, 2017, 2020; Carmo *et al.*, 2019; Martins *et al.*, 2019). In general, anuran species composition in restinga assemblages represents a subset of the species available in the forests that border the Brazilian coast (Rocha *et al.*, 2008; Silva *et al.*, 2008), though some species are apparently endemics of the restinga ecosystem (Carvalho-e-Silva *et al.*, 2000; Peloso *et al.*, 2012; Cardozo *et al.*, 2018).

In the Atlantic Forest of the state of Rio de Janeiro, 201 amphibian species are known to occur, including 197 anurans and four caecilians, with a high rate of endemism (Dorigo *et al.*, 2018). Ilha Grande is a large continental island at the southern coast of the state of Rio de Janeiro that is home to 34 anuran species (Rocha *et al.*, 2018). Most of the published data about the composition and structure of anuran assemblages in this island have originated from its forested areas (Rocha *et al.*, 2000, 2001, 2018; Van

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Sluys *et al.*, 2007; Bittencourt-Silva and Silva, 2013; Goyannes-Araújo *et al.*, 2015), which comprise most of the island's vegetation cover (Alho *et al.*, 2002). Herein, we compare the leaf litter anuran assemblages between forest and restinga habitats in Praia do Sul State Biological Reserve, on the southern coast of Ilha Grande, including data on species composition and relative abundance. We expected to record lower species richness and abundance of leaf litter anurans in restinga in comparison to the forest, since the restinga is an open and relatively xeric ecosystem characterized by sandy soils with high salt concentration (Franco *et al.*, 1984; Suguio and Tessler, 1984). In addition, we predicted that most leaf litter anuran species found in the restinga would also occur in the forest.

Ilha Grande is a continental island of ca. 19,000 ha on the southern coast of Rio de Janeiro State, southeastern Brazil. The present study was carried out within the Praia do Sul State Biological Reserve (Reserva Biológica Estadual da Praia do Sul, hereafter RBEPS; 3,502 ha), located at the seaward side of the island, with fieldwork being done in both forest (23° 10' 25"S, 44° 18' 45" W) and restinga (23° 10' 29" S, 44° 17' 56" W) habitats (see Rocha *et al.*, 2018). The climate on Ilha Grande is wet and warm, with average annual temperature of 21°C and annual rainfall of 2,200 mm (INEA, 2013).

Surveys were carried out in forest (at 10-100 m a.s.l.) and restinga (near sea level) areas during the wet season (September and October 2012 and October 2013), to eliminate potential differences caused by seasonal effects. Habitats were not sampled simultaneously, that is, our team sampled the habitats on alternate days in each field expedition. We sampled leaf litter frogs using 4 x 4 m quadrats (plot sampling; Jaeger and Inger, 1994), located at least 50-m apart, using 40 plots (quadrats) in each habitat type (640 m^2), totaling 1280 m^2 of sampled area. Total number of plots was divided between years (20 plots by habitat in each year) and none of the plots was established at exactly the same sampling point of a previous one. The corners of each plot were marked with wooden stakes and the area was enclosed with a 75 cm high soft mesh fence that was buried into or attached to the ground. After sunset, 3-4 people carefully searched each plot on their hands and knees (side by side) for about half an hour using headlamps. During the searches, leaves, branches and stones were overturned using hand rakes, and rock crevices and fissures among tree roots were also checked for frogs.

All anurans encountered within a given plot were identified to species level, weighed (to the nearest 0.2 g using Pesola®) and most of them were released after the crew finished searching the area. Individuals collected during the study were euthanized with a topical anesthetic gel (lidocaine 5%), fixed in 10% formalin solution, and preserved in 70% ethylene alcohol. We estimated the overall density of anurans per unit of forest floor (frogs/100 m^2) by dividing the total number of anurans found by the total area sampled in each habitat (640 m^2) and multiplying the result by 100 (see Allmon, 1991). The total biomass (g) and the biomass of leaf litter anurans per hectare (g/ha) were also extrapolated. We followed Haddad et al. (2013) to define each anuran species' reproductive mode.

The comparison of species richness between areas was undertaken by inference of overlapping 95% confidence intervals (MacGregor-Fors and Payton, 2013) for individual-based rarefaction curves performing 1,000 randomizations without replacement using the program EstimateS 9.1.0 (Colwell, 2013). Moreover, we compared alpha diversity measures between areas using "true diversity" (Jost, 2006) computed in the R package *hillR* (Li, 2018), using Shannon entropy. We calculated Hill-Shannon diversity considering that it responds strongly to both very high and very low rarity values, emphasizes neither rare nor common species, and has been considered a good choice for characterizing variation in biodiversity (Roswell *et al.*, 2021). Species composition similarity between areas was quantified through the Sørensen index (Magurran and McGill, 2011).

Voucher specimens of all anuran species were deposited at the amphibian collection of Museu Nacional (MNRJ), Universidade Federal do Rio de Janeiro (Appendix 1).

We recorded nine leaf litter anuran species during the study at the RBEPS, with eight of them being recorded in forest and six in restinga (Table 1; Fig. 1). The nine species recorded by us represent half of the 18 species known to occur at the RBPS (see Rocha *et al.*, 2018). Most of those species have direct development with terrestrial eggs or use lentic water bodies for reproduction (Nunes-de-Almeida *et al.*, 2021). Most of the species that we did not record were either arboreal or rheophilic, and thus unlikely to be found on the leaf litter. However, two of the unrecorded species (*Ischnocnema* cf. *guentheri*

Table 1. Reproductive modes (RM), number of individuals (NI), estimated density (frogs/100m2; between parenthesis), total biomass (g) and biomass per hectare (g/ha) recorded for each species of anuran recorded in the leaf litter of two Atlantic Rainforest areas (forest and restinga) within the Praia do Sul State Biological Reserve, Ilha Grande, in southeastern Brazil. Reproductive modes (according to Haddad and Prado 2005): 1 = Eggs and exotrophic tadpoles in lentic water; 2 = Eggs and exotrophic tadpoles in lotic water; 6 = Eggs and exotrophic tadpoles in water in tree holes or aerial plants; 8 = Eggs and endotrophic tadpoles in water in tree holes or aerial plants; 11 = Foam nest floating on pond; exotrophic tadpoles in ponds; 23 = Direct development of terrestrial eggs; 28 = Foam nest on the humid forest floor; subsequent to flooding, exotrophic tadpoles in ponds.

Species	Forest			Restinga		Total NI (frogs/100m2)
	RM	NI (frogs/100m2)	B (g)(g/ha)	NI (frogs/100m2)	B (g)(g/ha)	
Brachycephalidae						
Ischnocnema bolbodactyla (Lutz, 1925)	23	1 (0.2)	0.3 (4.7)			1 (0.1)
Ischnocnema parva (Girard, 1853)	23	39 (6.1)	11.6 (181.3)	15 (2.3)	5.9 (92.2)	54 (4.2)
Bufonidae						
Dendrophryniscus lauroi Miranda-Ribeiro, 1926 *a*	8	1 (0.2)	0.1 (1.6)	15 (2.3)	2.8 (43.8)	16 (1.3)
Rhinella ornata (Spix, 1824)	1/2	1 (0.2)	9.1 (142.2)			1 (0.1)
Craugastoridae						
Haddadus binotatus (Spix, 1824)	23	5 (0.8)	13.9 (217.2)			5 (0.4)
Hylidae						
Scinax sp. (gr. *perpusillus*)	6			1 (0.2)	0.1 (1.6)	1 (0.1)
Leptodactylidae						
Adenomera marmorata Steindachner, 1867	32	22 (3.4)	12.6 (196.9)	5 (0.8)	2.7 (42.2)	27 (2.1)
Physalaemus signifer (Girard, 1853)	11/28	4 (0.6)	5.7 (81.9)	1 (0.2)	1.0 (15.6)	5 (0.4)
Microhylidae						
Chiasmocleis lacrimae Peloso, Sturaro, Forlani, Gaucher, Motta and Wheeler, 2014	1	2 (0.3)	1.0 (15.6)	10 (1.6)	4.7 (73.4)	12 (0.9)
Total		75 (11.7)	54.3 (848.4)	47 (7.3)	17.2 (268.8)	122 (9.5)

a – Listed in Rocha et al. (2018) as *Dendrophryniscus brevipollicatus* (see Cruz *et al.*, 2019).

and *Leptodactyus flavopictus*) are actually ground-dwellers, and their absence in our plot samples may suggest that they are locally rare.

Individual-based rarefaction curves from forest and restinga showed that species richness of restinga fell within the 95% confidence interval of expected species richness of forest based on random subsamples of 47 individuals (Fig. 2), rejecting the first of our predictions, i.e., that the forest assemblage would be richer than that of the restinga. The forest assemblage had strong dominance by a small number of species and a long 'tail' of rare species, whereas the restinga assemblage had minor variation in rarity, and lower species richness (Fig. 3). We found a lower value of true diversity for the forest assemblage (3.9) than for the restinga assemblage (4.8). Diversity metrics summarize rank-abundance distributions to enable quantitative comparisons; in this way, true diversity measures the mean rarity of the species in the sample, and the community consisting of species that are, on average, rarer has higher diversity (Roswell *et al.*, 2021).

Index of similarity between forest and restinga was 0.7, with five shared species (Table 1). The species recorded in only one habitat type presented low abundances [forest: *Ischnocnema bolbodactyla*, N = 1; *Rhinella ornata*, N = 1; *Haddadus binotatus*, N = 5; restinga: *Scinax* sp. (gr. *perpusillus*), N = 1]. As we expected, almost all species recorded in the restinga were also sampled in the forest (with only one exception). Indeed, anuran species recorded in restinga areas usually have a wide geographic distribution and tend to also occur in other ecosystems of the Atlantic Forest domain (Carvalho-e-Silva *et al.*, 2000; Van Sluys *et al.*, 2004; Bastazini *et al.*, 2007). *Scinax* sp. (gr. *perpusillus*) was found by us only in the restinga,

Figure 1. Individuals representing the nine species recorded during fieldwork within the Praia do Sul State Biological Reserve, Ilha Grande, in southeastern Brazil. A) *Ischnocnema bolbodactyla*; B) *Ischnocnema parva*; C) *Dendrophryniscus lauroi*; D) *Rhinella ornata*; E) *Haddadus binotatus*; F) *Scinax* sp. (gr. *perpusillus*); G) *Adenomera marmorata*; H) *Physalaemus signifer*; I) *Chiasmocleis lacrimae*. Photos by F. B. S. Telles (A, F) and M. Santos-Pereira (B, C, D, E, G, H, I).

and it has also been recorded in other coastal areas near the RBEPS (Rocha *et al.*, 2018), probably being a species typical of that type of habitat. Because most anurans found by us at low altitudes inside the forest generally have direct development or use lentic water bodies for reproduction (Haddad and Prado, 2005; Nunes-de-Almeida *et al.*, 2021), these species can find favorable microclimatic conditions available on the leaf litter of restinga environments (e.g., Telles *et al.*, 2012; Carmo *et al.*, 2019). The availability of spawning sites, especially wetland habitats, is known to affect species composition in restinga areas (Oliveira *et al.*, 2017).

Despite *Ischnocnema bolbodactyla*, *Rhinella ornata* and *Haddadus binotatus* having been found only in the forest during our study, they have also been recorded in some open areas at Ilha Grande (Rocha *et al.*, 2018), and in other restinga areas of Southeastern Brazil (e.g., Carvalho-e-Silva *et al.*, 2000; Van Sluys *et al.*, 2004; Telles *et al.*, 2012; Carmo *et al.*, 2019). Thus, because these species had low abundance (or low detectability) locally, especially *I. bolbodactyla* and *R. ornata*, and both studied areas constitute an environmental continuum, the similarity of leaf litter anuran species composition between habitats at the RBEPS is probably even greater than our estimates suggest.

In the forest, 75 individual frogs were sampled, with *Ischnocnema parva* (N = 39; density = 6.1 ind/100 m^2) and *Adenomera marmorata* (N = 22; density = 3.4 ind/100 m^2) being the most abundant species, together accounting for *ca*. 80% of the individuals recorded in that habitat. In another forest area on Ilha Grande, at the Pico do Papagaio mountain, these two species were also the most common, representing *ca*. 65% of the individuals sampled in the leaf litter (Goyannes-Araújo *et al.*, 2015). In forested areas near the Dois Rios village, these species were also among the most abundant leaf litter anurans, together with *Brachycephalus didactylus* and *Cycloramphus* (= *Zachaenus*) *parvulus* (Rocha *et al.*, 2001; Van Sluys *et al.*, 2007). These anurans may be more widely distributed within the forest in Ilha Grande because they are not dependent on water bodies for reproduction (Haddad and Prado, 2005; Haddad *et al.*, 2013). For example, individuals of *A. marmorata* make foam nests in subterranean constructed chambers, where eggs are deposited and endotrophic tadpoles complete development (Haddad and Prado, 2005). The genus *Ischnocnema* belongs to the Brachycephaloidea superfamily (*sensu* Padial *et al.*, 2014), whose members lay eggs on the forest floor and have direct development. Direct-developing anurans are abundant in the leaf litter community of different areas in Neotropical leaf litter anuran assemblages (e.g., Scott 1976; Lieberman 1986; Fauth *et al.*, 1989; Giaretta *et al.*, 1999), possibly due to their independence from water bodies for reproduction.

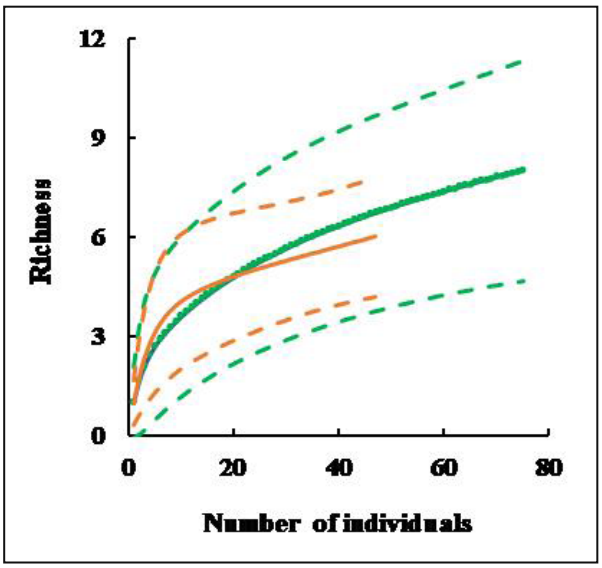

Figure 2. Individual-based species-rarefaction curves, with respective 95% confidence intervals (dotted lines), of two leaf litter anuran assemblages sampled in forest (in green) and restinga (in orange) within the Praia do Sul State Biological Reserve, Ilha Grande, in southeastern Brazil.

In the restinga, 47 individuals were collected, with *I. parva* and *Dendrophryniscus lauroi* (N = 15; density = 2.3 ind/100m^2 in both cases) as the most abundant species, followed by *Chiasmocleis lacrimae* (N = 10; density = 1.6 ind/100m^2). *Ischnocnema parva* (a species with direct development and terrestrial eggs) and *D. lauroi* (which deposits eggs in phytotelmata, where endotrophic tadpoles develop; Cruz *et al.*, 2019) comprised *ca*. 65% of all anurans sampled in that environment. Phytotelm-breeding anurans such as *D. lauroi* occur mainly in bromeliads, which are important in the restinga ecosystem as they are used as shelter, feeding, breeding, and courtship sites for several anuran species (e.g., Bastazini *et al.*, 2007; Silva *et al.*, 2008; Telles *et al.*, 2012). Bromeliads are common and widely distributed in the restinga at RBEPS (Araújo and Oliveira, 1988), which explains the relatively high local abundance of *D. lauroi*. During our study, we found *D. lauroi* and the treefrog *Scinax* sp. (gr. *perpusillus*) in the leaf litter, but we do not know if they were actually dwelling on the forest floor (e.g., for foraging) or

were just moving between perches.

The total biomass of leaf litter anurans in the forest was 54.3 g (extrapolated anuran biomass per hectare = 848.4 g/ha), with *H. binotatus* (13.9 g; 217.2 g/ha), *A. marmorata* (12.6 g; 196.9 g/ha) and *I. parva* (11.6 g; 181.3 g/ha) presenting the greatest biomass (Table 1). The total biomass of leaf litter anurans in the restinga was 17.2 g (extrapolated anuran biomass per hectare = 262.8 g/ha), with *I. parva* (5.9 g; = 92.2 g/ha) and *Chiasmocleis lacrimae* (4.7 g; = 73.4 g/ha) presenting the greatest biomass values (Table 1).

The estimated anuran density in the forest floor was high compared to some other studies of leaf litter-frog assemblages from various Atlantic Forest areas in Rio de Janeiro State (e.g., Rocha *et al.*, 2007, 2011, 2013; Almeida-Gomes *et al.*, 2008, 2010; Siqueira *et al.*, 2011a). Although the density estimates were near between the two types of habitats at the RBEPS (total density of 11.7 ind/100 m^2 in forest and of 7.3 ind/100m^2 in restinga), the estimated anuran biomass per unit of restinga (262.8 g/ha) was almost one-third of that of the forested area (848.4 g/ha). This may indicate that the forest assemblage tends to contain more larger individuals than that of the restinga, although more data are needed to confirm this. The estimated anuran biomass per unit of area in forest ecosystems from other sites of Rio de Janeiro State, in the municipalities of Guapimirim (938.4 g/ha; Rocha *et al.*, 2011) and Cachoeiras de Macacu (684.2 g/ha; Siqueira *et al.*, 2009), as well as another area in Ilha Grande (1150 g/ha; Rocha *et al.*, 2001), was close to what we found in the forest at RBEPS (848.4 g/ha). The latter value was also very similar to the one estimated by for an Atlantic Forest area in the state of Paraná, in southern Brazil (842.4 g/ha), although the estimated frog density in that area was only 3.7 ind/100m^2 (Santos-Pereira *et al.*, 2011).

All species found during our study are considered Atlantic Forest endemics, which was expected given the high degree of endemism for anurans in this biome (Haddad *et al.*, 2013; Rossa-Feres *et al.*, 2017). The record of *Scinax* sp. is referable to an apparently undescribed species of the *S. perpusillus* group (Bittencourt-Silva and Silva, 2013; Rocha *et al.*, 2018), which has so far been only reported from Ilha Grande and is possibly endemic to that island. In terms of conservation, most species recorded at the RBEPS are categorized as Least Concern (IUCN, 2021), except for *Chiasmocleis lacrimae*, which is considered "Endangered" (Pimenta and Peixoto, 2004). However, this species may need an update in its conservation status, as it has not been reassessed for more than 15 years and, since then, its northernmost populations have been found to represent distinct species (Tonini *et al.*, 2014; Forlani *et al.*, 2017), thus reducing the extent of the geographic distribution of *C. lacrimae*. None of the species found in the present survey is listed as threatened in the Red Book of Threatened Brazilian Fauna (ICMBio/MMA, 2018).

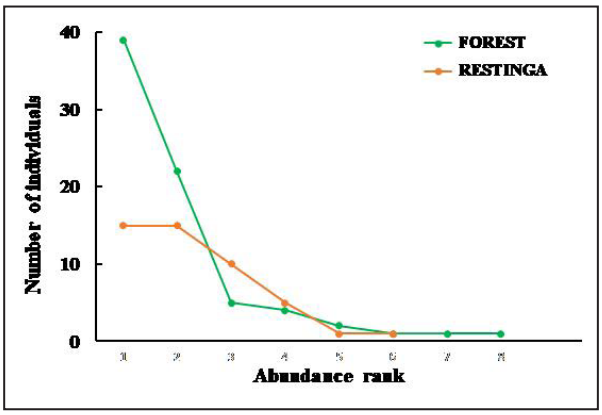

Figure 3. Observed rank-abundance distributions for the frog samples from two habitats (forest, in green, and restinga, in orange) within the Praia do Sul State Biological Reserve, Ilha Grande, in southeastern Brazil.

Although we have found higher absolute values of species richness in forest than in restinga, both rarefaction curves and true diversity indexes showed that the restinga assemblage contains a relatively high anuran species diversity. Anuran biomass production was more than three times smaller in restinga than in forest, which may suggest that productivity does not explain differences in species diversity between habitats, and that abiotic factors probably mediate the positive interrelationship between species diversity and ecosystem functioning.

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Appendix I

Voucher specimens of species collected in the present study deposited at the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ).

BRACHYCEPHALIDAE: *Ischnocnema bolbodactyla*: MNRJ 89141; *Ischnocnema parva*: MNRJ 93869-72, 93882. BUFONIDAE: *Dendrophryniscus lauroi*: MNRJ 93863-66, 93880; *Rhinella ornata*: MNRJ 93876. CRAUGASTORIDAE: *Haddadus binotatus*: MNRJ 93867-68, 93881. HYLIDAE: *Scinax* sp. (gr. *perpusillus*): MNRJ 93883. LEPTODACTYLIDAE: *Adenomera marmorata*: MNRJ 93860-62, 93878-79; *Physalaemus signifer*: MNRJ 93873-75. MICROHYLIDAE: *Chiasmocleis lacrimae*: MNRJ 93884-90.