Trabajo

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Composition, richness, abundance, and association of anuran fauna with the flooded habitats in the Ariri district, eastern Amazon

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ABSTRACT
Local inventories provide primary key information on diversity and distribution of species for conservation purposes. Here we describe the composition and conservation status of anuran fauna in flooded habitats eastern Amazon and evaluate to preference and the level of association of species with the flooded habitats in a poorly known area of the flooded forest present in the Ariri district, state of Amapá, North Brazil. Four nocturnal sampling events were carried out during the rainy season: beginning of the rainy season (March-May 2014 and December 2014-February 2015), mid rainy season (June-August 2014), and end of rainy season (September-November 2014), using visual encounter survey. We identified 29 anuran species. According to flooded habitat preference, the highest richness of species was found in high várzea (22 species, 75.9%, *H'* = 3.091), and 16 species (55.2%) was recorded with high association with flooded habitats. Including all flooded habitats, a greater number of constant species were found, followed by accessory and accidental species. Species accumulation curve showed a tendency toward stabilization of species richness only in the end of rainy season. Our study provides important data on the local anuran fauna and the presence of species typical of flooded and non-flooded habitats demonstrates a certain degree of similarity between species composition, reinforcing the importance of flooded habitats for the preservation of anurans of the Amazonia Forest in north Brazil.

Key words: Anurans; Inventories; Floodplain forests; Conservation; Natural history; Biology.

Introduction

Amazonian flooded forests are riverside areas of high rates of productivity that are flooded during the rainy season (Junk *et al.,* 2012), playing a key role in the regional biodiversity (Ramalho *et al.,* 2018). Representing between 3-4% of the Amazon basin, floodplains include a mosaic of habitats, ranging from the lotic mainstem river to slow-moving channels, ponds, and seasonally flooded fields and forests (Ward *et al.,* 2002). These habitats provide sites for aquatic and semi-aquatic taxa, including invertebrates and vertebrates, that exhibit adaptations and life cycles synchronized to match seasonal flood pulses (Ocock *et al.,* 2014).

This might be especially true for anurans. Many species that inhabit flooded forests (Ramalho *et al.,* 2016; Moraes *et al.,* 2022) in one or more stages of their life cycle, are usually present fidelity to their habitats regulated by the flood pulse (Ramalho *et al.,* 2018). However, despite the ecological importance of Amazonian flooded forests, these habitats are globally threatened by deforestation, fires, hydroelectric dam, invasive species, and pollution (Tockner and Stanford, 2002; Fearnside *et al.,* 2021). The maintenance of these habitats is one of the primary factors determining the presence of anuran species with adaptations to survive in this type of flooded forest and who need these environments to complete the reproductive cycles (Duellman and Trueb, 1994).

The flooded habitats present in the eastern Amazon are poorly known area in the Brazilian

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Amazonia, with several gaps in the knowledge of richness, composition, and geographical distribution of anuran fauna. Some studies on anurans in flooded areas have already been made in Central Amazonia (Waldez *et al.*, 2013; Ramalho *et al.*, 2016; Debien *et al.*, 2019; Moraes *et al.*, 2022), however, these studies in the eastern Amazon are largely underestimated and scarce (Corrêa *et al.*, 2015; Corrêa *et al.*, 2020), with several studies concentrated only in upland forest "*terra firme*" and protected areas (Benício and Lima, 2017; Silva e Silva and Costa-Campos, 2018; Pedroso-Santos *et al.*, 2019; Costa-Campos *et al.*, 2022). In the present study we investigated the composition and conservation status of anuran fauna in flooded habitats in eastern Amazon and evaluated the preference and the level of association of species with the flooded habitats.

Materials and methods

2.1. Study site

The study was undertaken riverside communities known locally as Ariri (0°17'57"N, 51°7'47"W) located north of the municipality of Macapá, Amapá state, in the eastern Brazilian Amazon. The area is flooded by black-water river of the Matapi river basin and is composed of Amazonian savanna with large areas of gallery forest and flooded forest (Silva *et al.*, 2016). The climate is Equatorial (Am) following Koppen's classification (Alvares *et al.*, 2013). The annual accumulated rainfall was 2261.6 mm. with an annual average temperature ranged of 24 °C to 32.1 °C (NHMET, 2022). The region is going through an intense urbanisation process and anthropogenic occupation, whereby most of its forest cover was affected.

2.2. Field procedures

Three sampling sites were selected for data collection. In each study site, a 200 m rectangular transect were built starting at a random point (Rödel and Ernst, 2004).

Equal numbers of transects (6) were surveyed during the diurnal (11:00-16:30h), crepuscular (18:00-19:00h) and nocturnal (19:30-22:00h) periods (see Fig. 1).

Each transect was searched for five days each during the rainy season: beginning of the rainy season (March-May 2014 and December 2014-February 2015), mid rainy season (June-August 2014), and end of rainy season (September-November 2014).

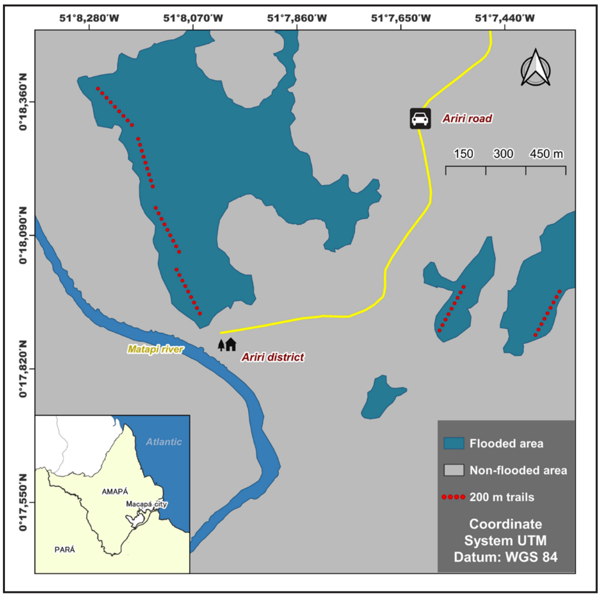


Figure 1. Map showing the study site in the Ariri district, eastern Amazon, municipality of Macapá, Amapá state, Brazil. An illustration of how transects were laid out. Illustrated is the 50 m. distance between the beginning of the first transect and the non-flooded edge.

The samplings were based on the Visual Encounter Survey (VES) method of Crump and Scott (1994), and Auditory Survey (Zimmerman, 1994), resulted in a total sampling effort of 460 person-hours.

The species were classified according to flooded habitat preference (Junk *et al.*, 2012) in aquatic macrophytes (habitats with presence of aquatic vegetation *Nymphoides indica* (L.) Kuntze and *Salvinia auriculata* Aubl), low várzea (habitats that spend much of the year flooded), high várzea (habitats subjected to shorter flooding periods), and non-flooded (habitats no influenced by the flood pulse - upland forest "*terra firme*") and numbered according to the level of association with the flooded habitats in three groups (Moraes *et al.*, 2022): (1) Amazonian species with geographic distribution encompassing other habitats adjacent to the flooded forest; (2) Amazonian species absent or rare in Amazonian flooded forests; and (3) Amazonian species typical from the flooded forest.

2.3. Data analysis

We analyzed the distribution of the species abundance, using rank abundance curves or Whittaker plots (Whittaker, 1965). Species are ranked in descending order from the highest number to the lowest and then the species are plotted in sequence numbering from the highest to the lowest along the X-axis. The logged transformed number of individuals by using log10 format is plotted at the Y-axis (Magurran, 2011). The curve formed in the plot will follow either four main patterns of rank-abundance curve: log-normal, log-series, broken stick, and geometric series. We analyzed these plots in PAST 2.17 (Hammer *et al.,* 2001).

The frequency of species occurrence and flooded habitat preference was classified according to the constancy index (Dajoz, 1983), which allowed its presence to be considered constant (present in ≥ 50% of samples), accessory (present in 25% ≤ C ≤ 50% of samples), or accidental (present in ≤ 25% of samples).

We generated rarefaction curves based on abundance to assess our sampling efficiency and examine the differences in species diversity across different sampling periods using the iNEXT package of R version 4.3.1 (R Development Core Team, 2020). We plotted four rarefaction/extrapolation curves, with confidence intervals, corresponding to three orders (q = 0, 1, 2) of Hill numbers (Chao *et al.,* 2014) to compare amphibian species diversity between the four sampling periods (and combinations) using 95% confidence intervals based on a 200 bootstrap replications method. The importance of the abundance distribution increases with increasing Hill order. For q=0, the Hill number is the richness, for q=1, it is the (exponential) Shannon entropy and for q=2, it is the inverse Simpson index.

We also estimated species diversity using the Shannon–Wiener index, based on richness and the abundance of species found in each rainy season and flooded habitats. To test for differences among each rainy season and flooded habiats in species diversity of anurans, we performed one-way analysis of variance (ANOVA) coupled with Tukey post-hoc test, using the software PAST 2.17 (Hammer *et al.,* 2001).

Results

3.1. Species composition

We identified 29 anuran species that belong to 14 genera and six families: Aromobatidae (1 species); Bufonidae (3), Hylidae (15), Leptodactylidae (7), Microhylidae (1), and Pipidae (1) (Fig. 2; Fig. 3; Table 1). *Lysapsus bolivianus* was the most abundant species, representing 9.8% of all specimens collected, followed by *Leptodactylus leptodactyloides* (8.4%) and *Sphaenorhynchus carneus* (7.9%).

The Shannon–Wiener index in the study area was similar in each rainy season, ranging from *H'* = 2.534 in the beginning of the rainy season to *H'* = 2.957 in the end of rainy season (Table 1). Species diversity differed significantly among rainy season (ANOVA: $F_{5.36} = 4.74$; p = 0.004), with differences significant (p = 0.017; p = 0.001) between beginning of the rainy season (March-May 2014) and mid rainy season (June-August 2014), and mid rainy season (June-August 2014) and beginning of the rainy season (December 2014-February 2015), respectively.

For the flooded habitats, the higher values of the Shannon–Wiener index was at higher várzea (*H'* = 3.091) and low várzea (*H'* = 3.045) (Table 2). Species diversity differed significantly among flooded habitats (ANOVA: $F_{7.17} = 7.35$; p < 0.001), with differences being significant between aquatic macrophytes and low várzea (p = 0.002), and aquatic macrophytes and high várzea (p < 0.001).

According to flooded habitat preference, the highest richness of species was found in high várzea (22 species, 75.9%) followed by low várzea (21 species, 72.4%) (Fig. 4). The most species (16 species, 55.2%) was recorded with high association with flooded habitats (Table 2).

3.2. Constancy of occurrence index and rank abundance curve

According to the constancy of occurrence index, the presence of 17 species was constant (58.6%), seven were accessory (24.2%), and five (17.2%) should be considered accidental (see Table 1). Considering the flooded habitat preference, the registered species at low várzea and high várzea were constant (72.4% and 75.9%, respectively), in non-flooded was accessory (44.8%), and in aquatic macrophytes was accidental (27.6%).

The rank abundance curve of frogs shown the broken stick pattern ($\chi 2$= 15.1, *p*= 0.94). The presence of dominant species was detected in this curve, with many accidental species represented by singletons (single individual) and doubletons (two individuals). *Scinax fuscomarginatus* and *Pipa pipa* were the singletons and *Scinax x-signatus* and *Trachycephalus typhonius* were the doubleton (Fig. 5).

3.3. Species accumulation curve

Our abundance-based rarefaction curves appeared to reach an asymptote (Fig. 6), indicating that our overall sampling effort, in different seasons was sufficient and that additional species are expected with increased sampling. The results of Hill numbers rarefaction curves revealed that there were slight

Figure 2. Anuran fauna recorded in flooded forest in the Ariri district, eastern Amazon: A) *Allobates femoralis*; B) *Rhinella major*; C) *R.* gr. *margaritifera*; D) *R. marina*; E) *Dendropsophus haraldschultzi*; F) *D. leucophyllatus*; G) *D. walfordi*; H) *Boana lanciformis*; I) *B. raniceps*; J) *Lysapsus bolivianus*; K) *Pseudis paradoxa*; L) *Scinax boesemani*; M) *S. fuscomarginatus*; N) *S. garbei*; O) *S. ruber*.

Figure 3. Anuran fauna recorded in flooded forest in the Ariri district, eastern Amazon: A) *Scinax x-signatus*; B) *Sphaenorhynchus carneus*; C) *S. lacteus*; D) *Trachycephalus typhonius*; E) *Pseudopaludicola boliviana*; F) *Adenomera hylaedactyla*; G) *Leptodactylus fuscus*; H) *L. leptodactyloides*; I) *L. macrosternum*; J) *L. mystaceus*; K) *L. petersii*; L) *L. podicipinus*; M) *Elachistocleis helianneae*; N) *Pipa pipa*.

Table 1. Abundance and constancy of occurrence per species of anurans recorded in flooded forest in eastern Amazon, municipality of Macapá, Amapá state, Brazil, during the beginning of the rainy season (March-May 2014 and December 2014-February 2015), mid rainy season (June-August 2014), and end of rainy season (September-November 2014). The samplings were based on the Visual Encounter Survey (VES) and Auditory Survey (AS). Constancy index, Aci = Accidental; Ace = Accessory; Cons = Constant.

Family/Species	**March-May/2014**		**June-August/2014**		**September-November/2014**		**December/2014-February/2015**		**Constancy index**	**Total**
	VES	AS	VES	AS	VES	AS	VES	AS		
Aromobatidae										
Allobates femoralis (Boulenger, 1884)	2	1	0	0	0	0	0	0	Aci	3
Bufonidae										
Rhinella major (Müller & Helmich, 1936)	8	0	5	0	3	0	4	0	Cons	20
Rhinella gr. *margaritifera*	0	0	2	0	5	0	0	0	Ace	7
Rhinella marina (Linnaeus, 1758)	2	0	2	0	4	0	4	0	Cons	12
Hylidae										
Dendropsophus haraldschultzi (Bokermann, 1962)	0	0	4	0	2	0	0	0	Ace	6
Dendropsophus leucophyllatus (Beireis, 1783)	0	0	3	2	2	0	0	0	Ace	7
Dendropsophus walfordi (Bokermann, 1962)	9	0	5	0	9	5	8	0	Cons	36
Boana lanciformis (Cope, 1871)	0	0	6	0	4	0	0	0	Ace	10
Boana raniceps (Cope, 1862)	4	0	7	0	8	0	4	0	Cons	23
Lysapsus bolivianus Gallardo, 1961	5	2	12	4	8	3	10	4	Cons	48
Pseudis paradoxa (Linnaeus, 1758)	0	2	5	3	0	2	0	4	Cons	16
Scinax boesemani (Goin, 1966)	0	0	2	0	4	0	1	0	Cons	7
Scinax fuscomarginatus (A. Lutz, 1925)	0	0	0	0	0	0	1	0	Aci	1
Scinax garbei (Miranda-Ribeiro, 1926)	2	0	3	0	0	0	0	0	Aci	5
Scinax ruber (Laurenti, 1768)	5	0	3	0	7	0	2	0	Cons	17
Scinax x-signatus (Spix, 1824)	0	0	2	0	0	0	0	0	Aci	2
Sphaenorhynchus carneus (Cope, 1868)	17	1	13	2	4	0	2	0	Cons	39
Sphaenorhynchus lacteus (Daudin, 1800)	4	2	8	3	3	0	0	2	Cons	22
Trachycephalus typhonius (Linnaeus, 1758)	0	0	0	0	2	0	0	0	Aci	2
Leptodactylidae										
Pseudopaludicola boliviana Parker, 1927	0	0	18	0	9	8	2	0	Cons	37
Adenomera hylaedactyla (Cope, 1868)	6	2	3	3	5	0	3	0	Cons	22
Leptodactylus fuscus (Schneider, 1799)	6	1	15	7	5	0	2	2	Cons	38
Leptodactylus leptodactyloides (Andersson, 1945)	0	0	23	0	13	0	5	0	Cons	41

Leptodactylus macrosternum Miranda-Ribeiro, 1926	0	0	1	0	5	0	4	0	Cons	10
Leptodactylus mystaceus (Spix, 1824)	0	0	4	1	2	0	0	0	Ace	7
Leptodactylus petersii (Steindachner, 1864)	6	0	10	3	3	0	0	0	Cons	22
Leptodactylus podicipinus (Cope, 1862)	5	0	12	4	2	4	0	0	Cons	27
Microhilydae										
Elachistocleis helianneae Caramaschi, 2010	0	0	1	0	2	0	0	0	Ace	3
Pipidae										
Pipa pipa (Linnaeus, 1758)	0	0	1	0	0	0	0	0	Aci	1
Species richness	15	26	24	16	---	---				
Shannon-Wiener diversity (H')	2.534	2.918	2.957	2.538	---	---				

differences in observed species richness (q = 0) and diversity (q = 1 e q = 2) across sampling periods.

Considering all 29 species, the number of species ranged from 15 (March-May 2014) to 16 (December 2014-February 2015) at the beginning of the rainy season to 26 in the middle of the rainy season (June-August 2014), and 24 species at the end of the rainy season (September-November 2014) (Fig. 7). The greatest number of individuals (202) was recorded in the mid of the rainy season.

Discussion

The anuran fauna composition in the study area resembles those reported in other studies that were carried out in flooded forest areas in the Central Amazonia (e.g., Höld, 1977; Waldez *et al.,* 2013; Ramalho *et al.,* 2016; Moraes *et al.,* 2022). The data showed that the most representative families in terms of species richness were Hylidae and Leptodactylidae. This result is similar to those of other studies in areas of flooded forest and floating meadows (Upton *et al.,* 2014; Böning *et al.,* 2017). We found a high diversity index caused by a heterogeneously distributed of anurans among the different flooded habitats. This promotes a high species turnover along the flooding gradient and increases regional species diversity (Moraes *et al.,* 2022). Arboreal and cryptozoic species such as *Trachycephalus thyphonius* and *Elachistocleis helianneae* may be detected only when they aggregate for reproduction, after heavy rains. Similarly, species strongly associated with aquatic habitats, such as *Lysapsus bolivianus*, *Pseudis paradoxa*, *Sphaenorhynchus carneus*, *S. lacteus* and *Pipa pipa*, may be absent from areas that periodically dry out.

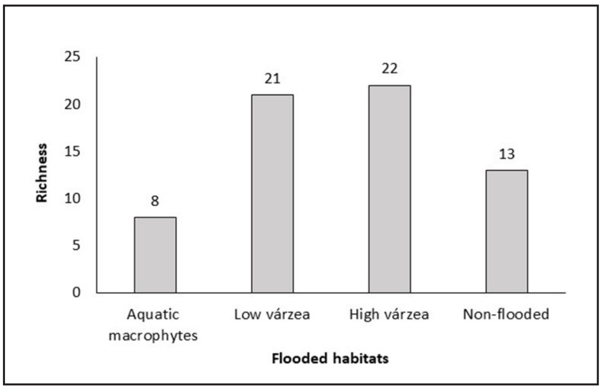


Figure 4. Richness of anuran fauna recorded in flooded forest in the Ariri district, eastern Amazon, according to flooded habitat preference.

Species abundance varied significantly between the rainy season, with most species being more abundant in the mid rainy season (June-August 2014), and end of rainy season (September-November 2014), months with lower precipitation. At these two seasons, although the accumulation of rain (256.1 mm) was twice lower than at the beginning of the rainy season (579.3 mm), the average temperature was higher. In general, anurans in neotropical regions tend to be more abundant in months with higher accumulation of rain and with higher temperatures, as they are clearly important factors driving the reproductive activity (Duellman and Trueb, 1994; Schalk and Saenz, 2016).

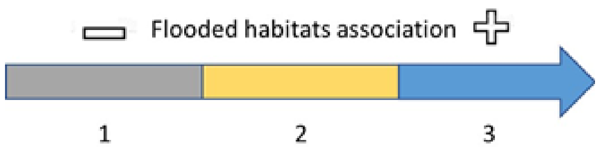
The higher abundance and the presence of anuran species exclusive recorded in the months of lower rainfall, might be related to the lek system found in almost all anuran assemblages, which consequently leads to a high aggregation of species and individuals (Wells, 2007). It is important to highlight that, despite having months with lower precipitation, the flooded habitats remain with water, which can favor the occurrence of anuran species not recorded in the periods with the highest rainfall. On the other hand, the higher abundance of anuran species may be related the influence of diversity structuring mechanisms on the anuran assemblages (Ramalho *et al.,* 2016), the high diversity of macrophytes (Mormul *et al.,* 2013) or the association between plant species and anuran species (Höld, 1977; Upton *et al.,* 2014).

All species considered abundant in the flooded habitats have a widely distributed across Amazonia (Frost, 2022). Except for *Adenomera hylaedactyla* and *Allobates femoralis*, all species were recorded exclusively in flooded habitats. This may be attributed to the association of these species to non-flooded (*terra firme* forests) that are not influenced by seasonal flood pulses (Waldez *et al.,* 2013; Ramalho *et al.,* 2016).

The index of constancy of occurrence showed that a low number of species classified as accidental, because the species *S. fuscomarginatus* and *Pipa pipa* were recorded only in one sample (singletons). Among the species considered accidental are those with explosive breeding (*T. thyphonius* and *E. helianneae*), which reproduce for a few days, often at high densities (Wells, 1977; Sousa and Costa-Campos, 2021), a dependent species of the non-flooded forest (*A. femoralis*), species recorded in association with the bromeliad (*S. fuscomarginatus*, *S. garbei* and *S. x-signatus*), and species strictly aquatic (*P. pipa*), which

Table 2. Flooded habitat preference per species of anurans recorded in flooded forest in the Ariri district, eastern Amazon, municipality of Macapá, Amapá state, Brazil. The colors show the level of association of anurans recorded with the flooded habitats.

Species	Flooded habitat preference			
	Aquatic macrophytes	Low várzea	High várzea	Non-flooded
Adenomera hylaedactyla (Cope, 1868)				gray
Allobates femoralis (Boulenger, 1884)				gray
Rhinella gr. *margaritifera*			gray	gray
Rhinella major (Müller & Helmich, 1936)			gray	gray
Rhinella marina (Linnaeus, 1758)		gray	gray	gray
Scinax ruber (Laurenti, 1768)		gray	gray	gray
Trachycephalus typhonius (Linnaeus, 1758)		gray	gray	gray
Leptodactylus fuscus (Schneider, 1799)			yellow	yellow
Leptodactylus macrosternum Miranda-Ribeiro, 1926		yellow	yellow	yellow
Leptodactylus mystaceus (Spix, 1824)			yellow	yellow
Pseudopaludicola boliviana Parker, 1927			yellow	yellow
Scinax fuscomarginatus (A. Lutz, 1925)			yellow	
Scinax x-signatus (Spix, 1824)			yellow	
Boana lanciformis (Cope, 1871)	blue	blue		
Boana raniceps (Cope, 1862)	blue	blue	blue	
Dendropsophus haraldschultzi (Bokermann, 1962)	blue	blue	blue	
Dendropsophus leucophyllatus (Beireis, 1783)		blue	blue	
Dendropsophus walfordi (Bokermann, 1962)		blue	blue	
Elachistocleis helianneae Caramaschi, 2010		blue	blue	
Leptodactylus leptodactyloides (Andersson, 1945)		blue	blue	blue
Lysapsus bolivianus Gallardo, 1961	blue	blue		
Leptodactylus podicipinus (Cope, 1862)		blue	blue	
Leptodactylus petersii (Steindachner, 1864)		blue	blue	
Pseudis paradoxa (Linnaeus, 1758)	blue	blue		
Scinax boesemani (Goin, 1966)		blue	blue	blue
Scinax garbei (Miranda-Ribeiro, 1926)		blue	blue	
Sphaenorhynchus carneus (Cope, 1868)	blue	blue	blue	
Sphaenorhynchus lacteus (Daudin, 1800)	blue	blue		
Pipa pipa (Linnaeus, 1758)	blue	blue		
Species richness	8	21	22	13
Shannon-Wiener diversity (H')	2.079	3.045	3.091	2.565



are more difficult to capture with the methodologies used in this study.

The results obtained from the rarefaction curve suggest that the species composition has stabilized in the end of rainy season (September-November 2014), indicating that sampling was sufficient to record most species present in the area, and that new sampling efforts are unlikely to add further species to this species composition. On the other hand, the rarefaction curves in the beginning and middle of the rainy season did not stabilize. These differences are related to the record of rare species in some samples (e.g., *Allobates femoralis*, *Pipa pipa*, *Scinax fuscomarginatus*, *S. garbei* and *S. x-signatus*), which

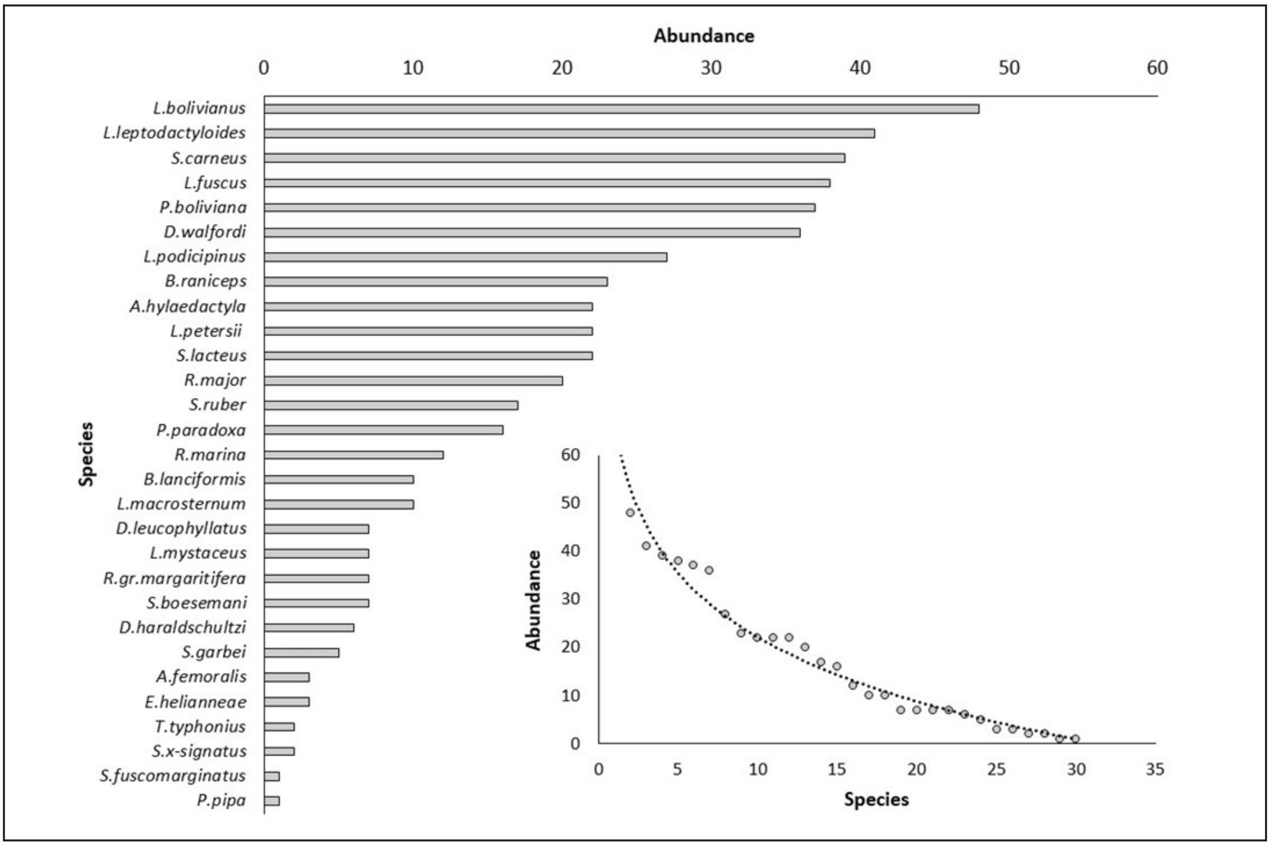


Figure 5. Whittaker diagram showing the relative abundance of the 29 anurans species recorded in flooded forest in the Ariri district, eastern Amazon. Bars represent relative abundance (%), numbers the total abundance of individuals of each species collected and observed.

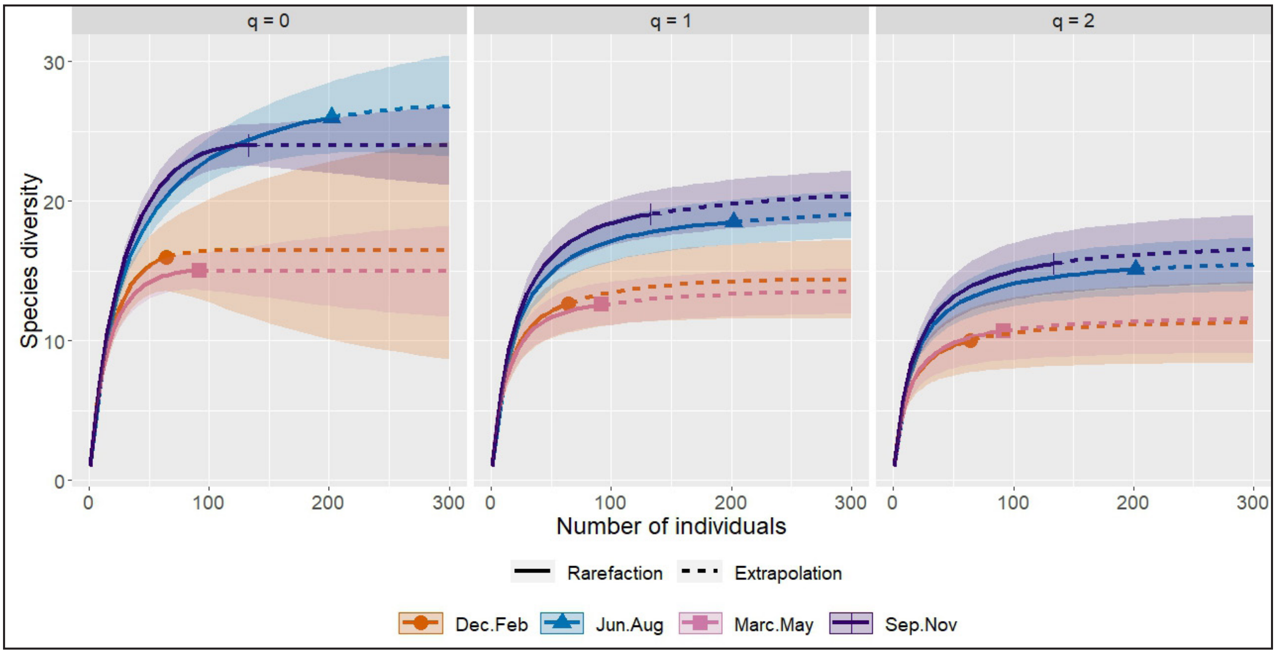


Figure 6. Comparison of the diversity of anuran species in different seasons through rarefaction solid lines and extrapolation dotted lines based on the number of individuals of the anurans species. Species diversity was estimated using Hill numbers: q = 0 (anuran species richness), q = 1 (exponential of Shannon's entropy index) and q = 2 (inverse of Simpson's concentration index).

agrees with the data found by Menin *et al.* (2008).

Although we could assess only the present-day pattern of species composition in the flooded habitats, the urbanization may have decreased species richness and abundance due to habitat degradation caused by anthropogenic activities (Fearnside, 2005), resulting in negligence in the protection and adequate conservation actions of the flooded habitats.

In this sense local inventories provide primary key information on diversity and distribution of species for conservation purposes, and the presence of species typical of flooded and non-flooded areas demonstrates a certain degree of similarity between species composition, reinforcing the importance of flooded habitats for the preservation of anurans of the Amazonia Forest in north Brazil.

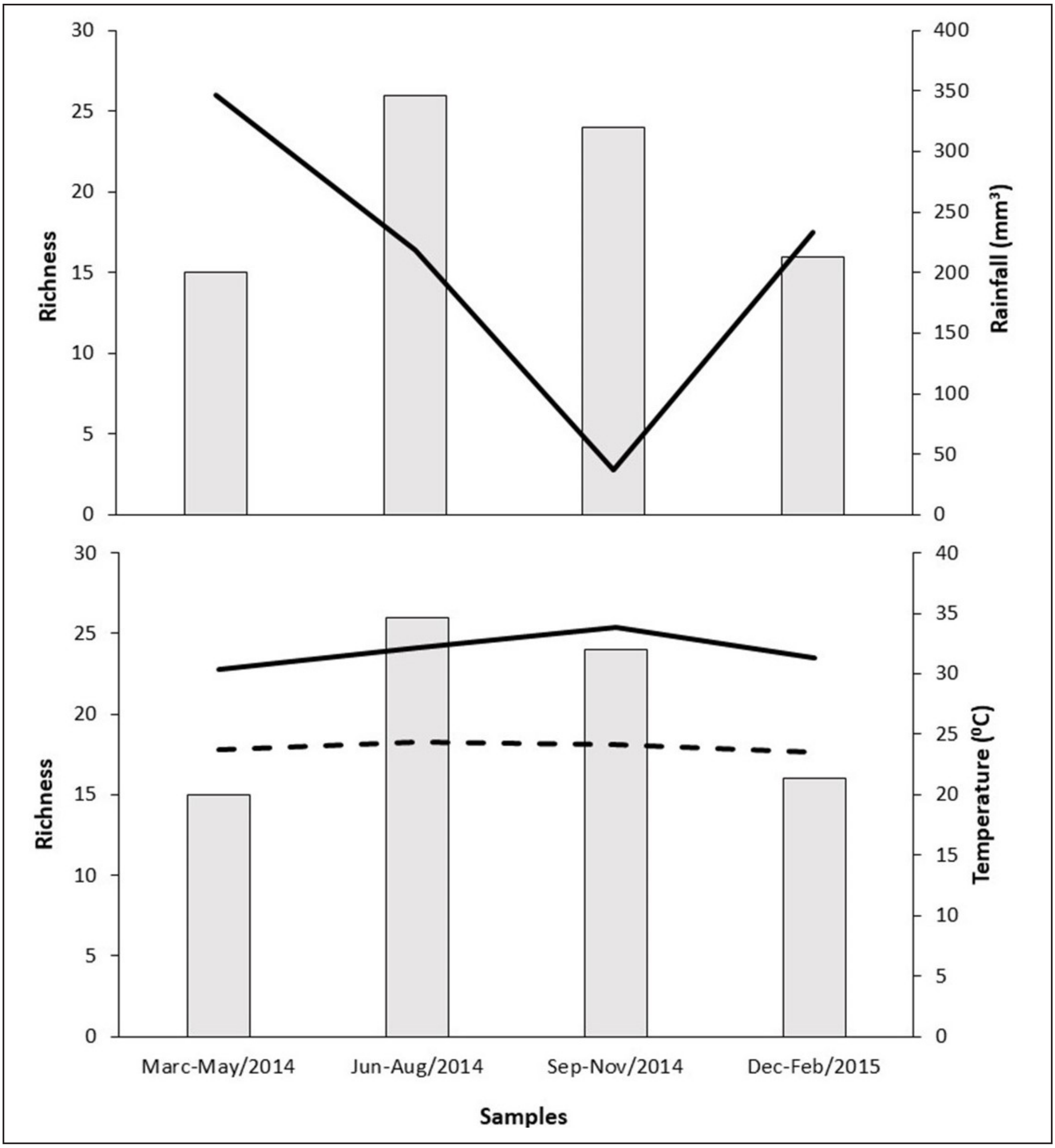


Figure 7. Correlation of anuran fauna richness recorded in an flooded forest in the Ariri district, eastern Amazon, with (A) rainfall (mm3) and temperature (°C).

Acknowledgments

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