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Anurofauna in fishponds in Rondônia state, Brazil

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Abstract: The study aimed to survey the temporal biodiversity and seasonal occurrence of anuran amphibians inhabiting fish farms. A total of seven fish farms were selected and visited during the two Amazon hydrological seasons: rainy and dry. Anuran amphibians were recorded through active searches conducted during their nocturnal vocalization period, between 6:00 and 10:00 p.m. A total of 151 specimens were observed in the rainy season and 164 in the dry season, representing 16 recorded species distributed across five families. The most abundant family was *Leptodactylidae*, with *Leptodactylus petersii* and *Leptodactylus fuscus* being the most abundant species. These species were commonly found on embankments, in fish pond supply channels, and in effluent areas. Regarding similarity in species composition per family, *Leptodactylidae* and *Hylidae* exhibited the most similar communities, with 40% and 30% similarity, respectively. There was a strong correlation (r = 0.92) between the abundance of Leptodactylidae and environmental factors such as average air temperature and rainfall. In contrast, weaker correlations were observed for *Hylidae* (r = 0.62) and *Bufonidae* (r = 0.44). Additionally, as rainfall increased, the abundance of *Bufonidae* decreased. Surveying anuran amphibian species in fish farms is an accessible method for assessing environmental quality. This study indicated that fish farms did not significantly influence the abundance or spatial and seasonal distribution of anuran amphibians. In other words, despite being artificial environments, these habitats were successfully colonized and adapted to by anurans.

Keywords
amphibian biodiversity; environmental quality; fishpond ecosystems; seasonal distribution
Palavras-chave
biodiversidade anfíbia; distribuição sazonal; ecossistemas de viveiros de peixes; qualidade ambiental

Resumo: O estudo teve como objetivo realizar um levantamento da biodiversidade temporal e da ocorrência sazonal de anfíbios anuros em pisciculturas. Foram selecionadas sete pisciculturas, visitadas durante as duas estações hidrológicas da Amazônia: chuvosa e seca. O registro dos anuros foi efetuado por meio de buscas ativas, conduzidas durante o período noturno de vocalização das espécies, entre 18h e 22h. No total, foram observados 151 espécimes na estação chuvosa e 164 na estação seca, correspondendo a 16 espécies registradas, distribuídas em cinco famílias. A família mais abundante foi Leptodactylidae, destacando-se Leptodactylus petersii e Leptodactylus fuscus como as espécies mais representativas. Esses anfíbios foram frequentemente encontrados em taludes, nos canais de abastecimento dos viveiros e nas áreas de efluentes. Quanto à similaridade na composição de espécies por família, Leptodactylidae e Hylidae apresentaram as comunidades mais semelhantes, com 40% e 30% de similaridade, respectivamente. Observou-se uma forte correlação (r = 0,92) entre a abundância de Leptodactylidae e fatores ambientais, como temperatura média do ar e precipitação pluviométrica. Em contrapartida, correlações mais fracas foram verificadas para Hylidae (r = 0,62) e Bufonidae (r = 0,44). Ademais, à medida que a precipitação aumentava, a abundância de Bufonidae diminuía. O levantamento de espécies de anfíbios anuros em pisciculturas configura um método acessível para a avaliação da qualidade ambiental. Os resultados deste estudo indicaram que as pisciculturas não exerceram influência significativa sobre a abundância, a distribuição espacial ou a ocorrência sazonal dos anuros. Em outras palavras, apesar de constituírem ambientes artificiais, esses habitats foram progressivamente colonizados e adaptados pelos anuros.

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Introduction

Population growth, urbanization, and poor water management have degraded aquatic ecosystems, affecting water quality and species (Lima-Pinheiro et al., 2023). Pollution from sewage, pesticides, and industrial waste has intensified this deterioration (Authman et al., 2015). The uncontrolled rise in chemical compounds has led to the anthropization of these environments, compromising environmental quality and threatening the health of living organisms (Silva et al., 2018).

Population growth, urbanization, industrial expansion, and poor water management have intensified environmental degradation, affecting water quality and aquatic species (Lima-Pinheiro et al., 2023). Aquatic ecosystems face progressive deterioration due to various chemical pollutants from domestic sewage, pesticide residues, and industrial waste carried by runoff (Authman et al., 2015). This has led to an uncontrolled rise in chemical compounds, causing the anthropization of these environments. Consequently, environmental quality declines, posing risks to the health of living organisms (Silva et al., 2018).

Amphibians are highly sensitive to environmental changes due to their permeable skin, limited mobility, complex life cycles, diverse reproductive strategies, and dietary differences between life stages (Silva et al., 2018; Gonçalves et al., 2019). The number of anuran species in the Amazon is underestimated, with limited studies revealing many new species. Research on Amazonian anurofauna remains scarce, particularly in artificial environments like fish farms.

Given the justifications, this study aimed to carry out a survey of the temporal diversity and seasonal occurrence of anuran amphibians that live in fish farms.

MATERIAL AND METHODS

Bioethical aspects

This study was approved by the Biodiversity Authorization and Information System (SISBIO) registration authorization number 84820-1, and with authentication code no. 0848200120220914.

Study area and climatological data

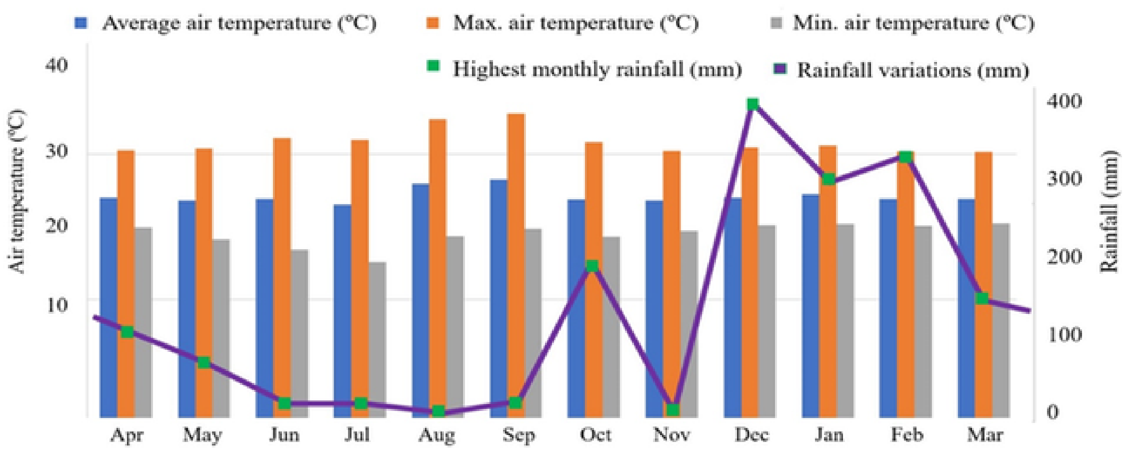
A total of 7 fish farms located in the Centro-Leste microregion of the Rondônia state were selected, more specifically located in the municipalities of Ji-Paraná (3 fish farms), Ouro Preto do Oeste (2 fish farms) and Teixeirópolis (2 fish farms) (Figure 1). These fish farms were visited in the two Amazon hydrological seasons, rainy (January to April) and dry (May to July) in the year 2023.

Figure 1 - Location of fish farms visited to survey anurofauna, Centro-Leste microregion of the Rondônia state, Brazil.

Source: map prepared by the authors.

According to CPTEC/INPE (2023), the climate of the Centro-Leste microregion of Rondônia state is classified in the Köppen system, as predominant type Am - Tropical Rainy Climate (Alvares et al., 2013). Being the climatological average of the air temperature, during the coldest month above 18 °C (megathermic) and a well-defined dry period during the dry period, when there is a moderate water deficit with rainfall levels below 50 mm/month. The average annual rainfall varies between 1,400 and 2,600 mm/year, while the monthly average air temperature varies between 24 and 26°C (Figure 2). In relation to the latest meteorological data, the months of October/2021 (200mm), December/2021 (189.50 mm), January/2022 (336.05 mm) and February/2022 (359.66 mm), showed the highest precipitation averages. While the months of August/2021 and September/2021, showed the highest average temperatures, >35º C, respectively. There were no significant variations in the other months (Figure 2).

Figure 2 - Monthly averages of rainfall (mm) and air temperature in the study region, in the different Amazonian hydrological seasons.



Source: CPTEC/INPE, 2023.

Data collected

The search for anuran amphibians was carried out by active search using the breeding site survey method (Scott Jr.; Woodward, 1994), during the species' nocturnal vocalization shift, between 6 and 10 pm. To complement the anurofauna survey, an active search for species was carried out, which consisted of an active search for amphibians in specific microhabitats, such as under logs and rocks, inside bromeliads and other refuges, as well as an aural search for species that vocalize and reproduce far away. of bodies of water, in leaf litter or perched in bushes and trees.

The abundance of the species was determined by the number of males vocalizing, considering the average of three estimates, carried out along the perimeter of each habitat. It is important to highlight that three estimates were considered to calculate abundance, estimates carried out along the perimeter of each habitat, the estimate with the greatest abundance was considered. To avoid sampling deviation, due to the species' vocalization shift, the sequence of visits to fish farms was randomized by draw.

Data analyzes

Species were recorded using images and recording vocalizations with a digital camera. In identifying the species, the Brazilian list of amphibians from the Sociedade Brasileira de Hepetologia (SBH) was used. From the information obtained in the field, it was possible to propose a list of anuran species for the study location and analyze their occurrence and occupation of microhabitats for each sampling area.

In temporal distribution, the seasonal occurrence of anuran species was analyzed based on vocalization records. This was possible by comparing parameters such as rainfall and average monthly temperature with the anuran species recorded monthly.

The similarity in species composition between the sampled areas was calculated using the Sörensen index (Lima et al., 2022), given by the form expressed in Equation (1).

$$IS = 2C/S1+S2 \qquad (1)$$

Where: C is the number of species common to both areas; S1 is the number of species in area P1 and S2 is the number of species in area P2. The index varies from 0 (maximum dissimilarity) to 1 (maximum similarity).

The richness of each species was estimated in all seven fish farms visited, based on the abundance of the two hydrological seasons, by extrapolation of the accumulation curves, the data were showed according to Abundance-based Coverage Estimator (ACE) (Miranda et al., 2015). Diversity in each fish farm was assessed by applying Shannon-Wiener diversity and Pielou equitability indices (Krebs, 1999), such as the logarithm of the total number of species at each sampling point (High – equal to or above 0.70 – H'>= 0.70 H'max). The influence of climate was considered (average air temperature and rainfall on species richness and abundance of male anurans in vocalization activity.

The data obtained were subjected to descriptive statistical analysis, means and standard deviation, abundance and frequency, in addition to analysis of variance of the means. Pearson correlations were performed to analyze the factors abundance of families in vocalization activity with environmental conditions - average air temperature and rainfall.

For statistical analyses, the RStudio Development Core Team software, version 3.5.3, was used.

Results

A total of 151 specimens were observed in the rainy season and 164 specimens in the dry season, from a total of 16 recorded species distributed in 5 families. The most abundant species of the Leptodactylidae family - *Leptodactylus petersii* and *Leptodactylus fuscus* (Figure 3 A), were found on the slopes and supply channels of the ponds and in the fish farming effluent. However, the most abundant species of the Hylidae family - *Hypsiboas multifasciatus, Hypsiboas fasciatus, Hypsiboas geographicus* and *Dendropsophus minutus* (Figure 3 B), were found in shrub vegetation and grasses (Table 1).

The anurans of the species *Leptodactylus (Lithodytes) lineatu*, Leptodactylus pentadactylus, *Hypsiboas lanciformis, Hypsiboas boans, Rhinella schneideri* and *Rhinella marina* were found only in the dry season, while the species *Pristimantis* fenestratus was found only in the rainy season (Table 1).

Figure 3 - Most abundant anuran amphibian species, (A) *Leptodactylus petersii* and *L. fuscus* (family Leptodactylidae and (B) *Hypsiboas multifasciatus,* H. *fasciatus,* H. *geographicus* and *Dendropsophus minutus* (family Hylidae).

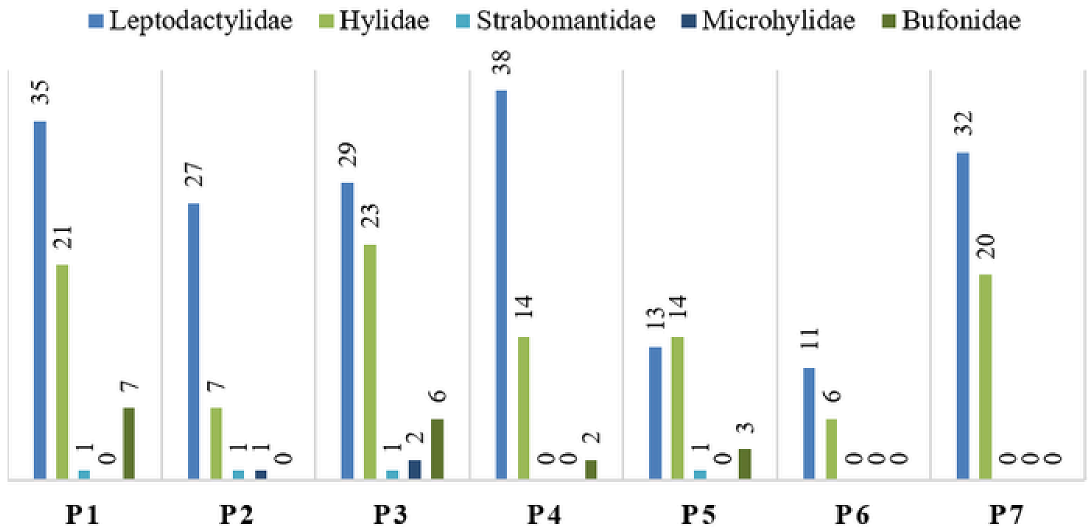
Table 1 - Inventory of anuran amphibian species recorded in fish farms. Vocalization sites (SV): embankments on fish ponds (Ta), supply channels (Ca), effluents (Ef) grasses (Gr) and shrub vegetation (Va).

Families	Species	Hydrological stations		SV
		Rainy	Dry	
Leptodactylidae	*Leptodactylus fuscus* (Schneider, 1799)	33	51	Ta, Ca, Ef
	Leptodactylus petersii (Steindachner, 1864)	41	54	Ta, Ca, Ef
	Leptodactylus pentadactylus (Laurenti, 1768)	0	3	Ca
	Leptodactylus (Lithodytes) lineatus (Schneider, 1799)	0	3	Ef
Hylidae	*Hypsiboas fasciatus* (Günther, 1859)	14	3	Va
	Hypsiboas lanciformis (Cope, 1870)	0	2	Va, Gr
	Hypsiboas multifasciatus (Günther, 1859)	23	3	Va
	Hypsiboas geographicus (Spix, 1824)	14	20	Va, Gr
	Dendropsophus minutus (Peters, 1872)	12	2	Ta, Va
	Scinax ruber (Laurenti, 1768)	6	4	Ta, Gr
	Hypsiboas boans (Linnaeus, 1758)	0	2	Va
Strabomantidae	*Pristimantis fenestratus* (Steindachner, 1864)	4	0	Ta, Va
Microhylidae	*Elachistocleis bicolor* (Guérin-Méneville, 1838)	2	1	Ta, Gr
Bufonidae	*Rhinella granulosa* (Spix, 1824)	2	7	Ta
	Rhinella schneideri (Werner, 1894)	0	6	Ta
	Rhinella marina (Linnaeus, 1758)	0	3	Ta, Gr

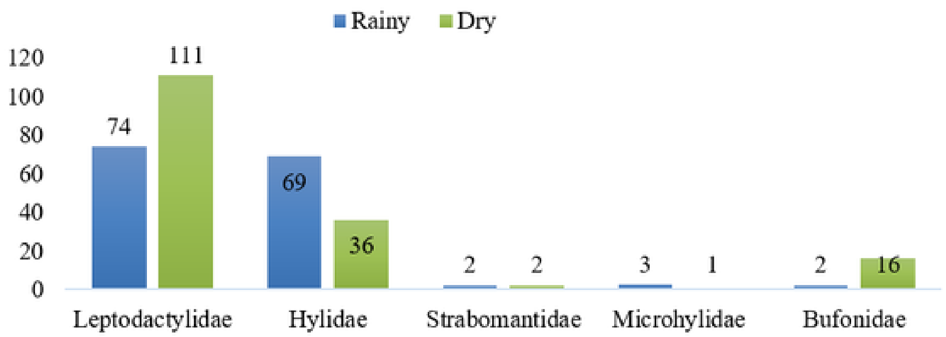
In relation to abundances, the families Leptodactylidae and Hylidae showed the highest abundances and the highest frequencies in fish farms (Figure 4 A). Furthermore, they were the most frequent families in the rainy and dry seasons (Figure 4 B), and in the dry season the Bufonidae family also showed a significant frequency (Figure 4 C).

Figure 4 - Abundances of anuran amphibian families by fish farms (A) and by hydrological seasons (B) and frequency of families (C).

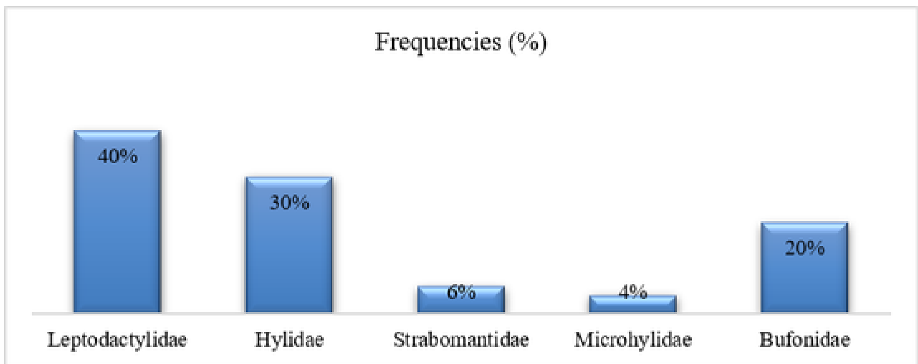
(A)



(B)

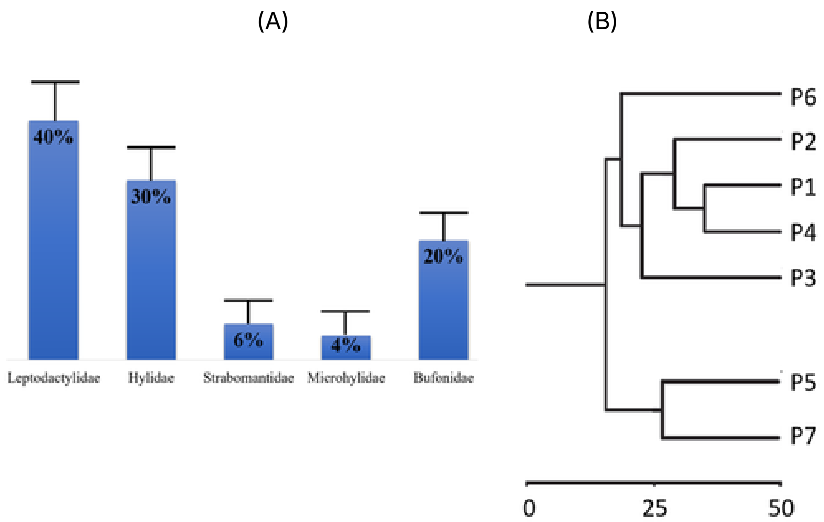


(C)



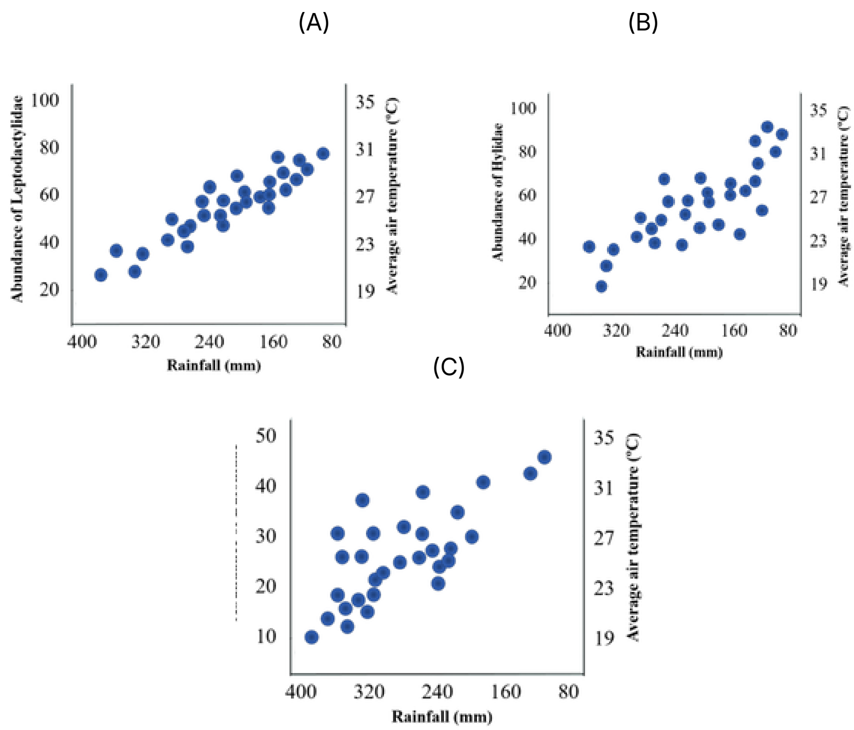
Regarding similarity analysis, considering the composition of species per family, the families Leptodactylidae and Hylidae showed the most similar communities (40 and 30%, respectively) (Figure 5 A). When analyzing by sampling point (by fish farms), the greatest similarity in the anurofauna composition was verified between P1 and P4 (IS > 0.70) (Figure 5 B).

Figure 5 - Similarity in the composition of anurofauna by family (A) and by sampling point (by fish farms (B).



As shown in Figure 6, there was a significant correlation (r= 0.92) between the abundance of the Leptodactylidae family and environmental conditions of average air temperature and rainfall. While for the Hylidae and Bufonidae families the correlations were weaker (r= 0.62 and 0.44), so that as rainfall increased the abundance of the Bufonidae family reduced.

Figure 6 - Pearson correlations between the abundance of the families Leptodactylidae (A), Hylidae (B) and Bufonidae (C) in vocalization activity with average air temperature and rainfall.



DISCUSSION

The water from fish farms has a high load of nutrients, concentrated P and N compounds, in addition to the possibility of the presence of toxic metals, cyanotoxins, mycotoxins, etc. (Terada-Nascimento et al., 2023). It is important to highlight that almost all fish farms visited in this study were at some level of eutrophication, in addition to the examples mentioned above, there are other contaminating factors, from agricultural and industrial influences to urban effluents near fish farms. Another issue worth mentioning is that fish farms in Rondônia state are very close to coffee and corn crops Câmara et al. (2015), surface and groundwater from rain enters nurseries through leaching, polluting agents (pesticides) from agriculture may be influencing biodiversity, such as fungicides, acaricides and other medicines for veterinary use (Barbosa et al., 2023).

Currently, more than 5 thousand anurans are known, Brazil has the greatest specific diversity of anuran amphibians, with more than 800 species described to date (SBH, 2021), where 60% of these are endemic, that is, exclusive to Brazil. Artificial environments such as cultivation ponds and urbanization close to forest fragments cause significant changes in the maintenance of anuran amphibian species, which makes it necessary to evaluate environmental conditions. Given this, Calderón et al. (2014) indicated observing and recording the richness and abundance of anuran amphibians as a metric for environmental impact studies. Furthermore, artificial environments impact ecosystems through habitat loss and fragmentation, loss of diversity, increased surface runoff and discharges of contaminants and invasion of exotic species. Therefore, Calderón et al. (2017) advised monitoring the richness and relative abundance of aquatic invertebrates and anuran amphibians as bioindicators of environmental quality, together with the study of physicochemical and microbiological analyzes of reservoir water.

According to Barbosa and Alves (2014), when investigating the diversity of anuran amphibian species in a fragment of the Atlantic Forest in Northeast Brazil and relating the individuals collected using the habitat structures and microhabitats found, the recorded anurofauna did not represent the real diversity of the Pau Ferro Forest region due to the accelerated anthropization of the region. The abundances and frequencies of the 21 species were overestimated, since the collection area is visibly more anthropized and smaller in area when compared to the legal size of the reserve.

When observing the results in the current study, in which the number of families and species recorded also do not represent the diversity of amphibians in this location in the Amazon basin, we share the same view as Barbosa and Alves (2014) in which human activities deeply compromise the ecosystems of the Amazonian anurofauna, especially when it comes to artificial fish pond environments that drastically compromise the vertical stratification of microhabitats. Conte and Rossa-Feres (2006) demonstrated interesting results and may explain the seasonal differences in family abundances in the current study. The authors observed that anuran families from more preserved forest areas have a greater diversity and rate of endemism of anuran amphibians. In surveys carried out by the authors over a few years in rainforests in the interior of the Paraná state, a total of 34 species from five families were recorded: Bufonidae, Hylidae, Leptodactylidae, Microhylidae and Ranidae. Conte and Rossa-Feres (2006) inferred that disturbances and reduction in abundance were gradually due to deforestation and other anthropic factors, most species vocalized during the rainy season and similarity in species composition was associated with the plant physiognomy of the sampled areas.

With the advancement of information technologies, the process of discovering biodiversity has been accelerated, and this is due to the use of molecular tools, mainly the 16S rRNA gene barcode, which is used to identify species and discover cryptic lineages. However, few anuran amphibians from the Amazon have molecular sequence data available in public databases, which contrasts with the considerable species richness of this biome. Motta et al. (2018) carried out a study of the occurrence of cryptic species using the mPTP delimitation algorithm. We morphologically identified 26 species, of which 23 were molecularly confirmed with the remaining three species identified as other congeneric species, as sequences with the same epithet do not exist in GenBank. Of these 23 recorded species, nine contained a lineage restricted to the Amazon basin.

Knispel and Barros (2009) developed a study aiming to inventory anuran species in three locations in the urban region of Altamira city, located in western Pará state. A total of 15 species were recorded during the study. The Hylidae family was the most represented, with eight species recorded. The data were compatible with the degree of conservation of the collection areas. While the study conducted by Souza, Souza and Morato (2008) monitored the abundance, richness and composition of anurofauna at different successional stages in and around a forest reserve in the state of Acre. A total of 27 species distributed in seven families were observed, with the greater richness in capoeiras and matrix environments being largely explained by the existence of water pools in these places, greater structural heterogeneity and the fact that they may constitute intermediate stages of disturbance. These stages have been identified as factors that promote and maintain high levels of biodiversity. Environments with intermediate levels of disturbance are important for the conservation of anurofauna.

Bernarde and Macedo (2008) carried out a survey of anurofauna on farms in the municipality of Espigão do Oeste, Rondônia state. A total of 1,324 specimens belonging to 27 species were captured, distributed across nine families: Aromobatidae, Brachycephalidae, Bufonidae, Cycloramphidae, Dendrobatidae, Hylidae, Leiuperidae, Leptodactylidae and Microhylidae. The use of interception and pitfall traps made it possible to record 57% of the species known for this location, being particularly efficient for capturing anurans with terrestrial and fossorial habits. The authors observed fewer specimens in the dry season and there was greater diversity during the rainy season. Furthermore, greater richness and diversity was recorded in forest environments compared to pasture, with a decrease in species with deforestation (anthropization).

Bernarde and Macedo (2008) stated that transforming the forest into pastures causes a reduction in places for reproduction, a decrease in food availability, loss of litter, soil compaction, microclimatic changes, an increase in the abundance of species in open areas, etc.) creating unfavorable conditions for the survival of anuran amphibians. However, in the case of the construction of fish farms in Rondônia state, in the current study indicates that apparently the anuran amphibians adapted to the artificial environment and started to occupy these new microhabitats without significant variations in abundances and spatial and seasonal distributions.

The need to carry out new faunal inventories in all Brazilian biomes is extremely urgent, especially in the Amazon basin, given its underestimation, vast extension and lack of inventories. Specifically in the state of Rondônia, this urgency is mainly due to the rapid loss of habitats suitable for the existence of anurans, such as the interior of vegetation and bodies of water.

Regarding ecological implications, the present study highlights that aquaculture ponds exhibit a high nutrient load, including P and N compounds, and may contain toxic metals, cyanotoxins, and mycotoxins (Sánchez-Parra et al., 2020). The eutrophication of these environments was a common factor observed in the visited ponds, exacerbated by contaminant sources such as stormwater runoff, agricultural residues, and industrial and urban discharges. The proximity of aquaculture farms to coffee and corn plantations in Rondônia may be influencing local biodiversity through the leaching of pollutants, such as pesticides and veterinary drugs (Barbosa et al., 2023).

The biodiversity of anuran amphibians in Brazil is considerable, with over 800 described species, of which 60% are endemic (SBH, 2021). However, artificial environments such as aquaculture ponds significantly alter the population dynamics and distribution of anurans, requiring a detailed assessment of environmental conditions. Previous studies recommend monitoring the richness and relative abundance of anurans and aquatic invertebrates as bioindicators of environmental quality. The impact of urbanization and habitat fragmentation on species maintenance is also a relevant concern (Potrich et al., 2020).

The results of the current study support the idea that human activities compromise anuran ecosystems in the Amazon, particularly in artificial environments that alter microhabitat stratification. Conte and Rossa-Feres (2006) demonstrated that anuran diversity and endemism are higher in preserved forest areas and that species composition is associated with vegetation physiognomy. Similarly, Bernarde and Macedo (2008) recorded greater anuran diversity in forest environments than in pasture areas, suggesting that deforestation reduces the availability of essential resources, such as breeding and feeding sites.

Despite these challenges, the study showed that anuran species were able to colonize aquaculture ponds without significant variations in abundance and spatial and seasonal distribution. This result contrasts with previous research indicating a reduction in biodiversity in impacted environments. However, the mechanisms that enable this adaptation still need to be further investigated, particularly regarding water quality and resource availability in these locations (Cunha et al., 2021). The advancement of information technologies has accelerated biodiversity discovery through molecular tools, such as 16S rRNA gene sequencing. However, most anuran species in the Amazon still lack available sequences in public databases, which hinders the identification of cryptic lineages and the understanding of the true diversity of the biome (Motta et al., 2018).

Conclusion

Surveying anuran amphibians in fish farms is a practical method for assessing environmental quality. This study found that fish farms did not affect species abundance or distribution, as anurans successfully colonized and adapted to these artificial habitats.

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