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ANIMAL SCIENCE

Testing the efficiency of passive acoustic monitoring and active monitoring in anuran community in lotic environments in Itinguçu State Park, Peruíbe-SP

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Abstract: The following work presents the first study applying the passive acoustic monitoring of anurans at lotic environments for a long time. This study aims to test the efficiency of the passive acoustic monitoring method and active monitoring in detecting anurans in lotic environments of Itinguçu State Park. Specifically, we tested whether species richness differs when comparing active and passive monitoring surveys. Therefore, this study aims to test the efficiency of the passive acoustic monitoring method and active monitoring in detecting anurans in lotic environments of the Itinguçu State Park. The passive acoustic monitoring period was 72 uninterrupted hours at each collection point with intervals of 45 days. Finally, species richness was calculated, and the efficiency of the methods was compared in different scenarios. Our results demonstrated that the park has species that vocalize day and night, but most at night, there is overlapping acoustic niche; waterfall environments harm the quality of recordings; and in lotic environments the active monitoring method was more efficient than the passive acoustic monitoring in all sampling scales. Although the passive acoustic monitoring was not as efficient in a low temporal scale, it tends to increase in efficiency with longer sampling duration.

Key words: passive acoustic monitoring, active monitoring, anuran, lotic environments.

INTRODUCTION

Anuran amphibians (toads, tree frogs and frogs) belong to the order Anura, which currently has 7632 species described around the world (Frost 2023). Most species are assigned to the Neotropical region, which comprises the tropics of the Americas and extends from central Mexico to Argentina including the Caribbean, already known for its remarkable diversity of taxonomic groups (Morrone 2014). Within this scenario, Brazil is home to the greatest wealth of anurans in the world, with 1188 recognized species, distributed in 20 families (Segalla et al. 2021). However, these represent the group of vertebrates that comprises more conservation threat categories (IUCN 2022), which makes taxonomy and ecology studies of these species fundamental and essential.

Anurans are characterized as tetrapod with a biphasic way of life (terrestrial and aquatic phase), having a generally thin integument that represents a respiratory surface that absorbs not only oxygen but also other substances from the environment in which they live, making them excellent indicators of environmental quality (Hickman et al. 2013). However, one of the most striking characteristics of anurans is their singing repertoire. The diversity of sounds produced shows us how communication works in animal societies, signals that can provide information about the identity, healthy and reason for that individual's vocalization (Llusia 2013). The repertoire of anurans calls is currently classified into three categories based on the social context in which they occur: (i) reproductive, (ii) aggressive, and (iii) defensive (Toledo et al. 2014). The most reliable type of call for taxonomic studies is the advertisement call produced by males in the breeding season, since it is the most recorded and specific to each species as it involves attracting females (Köhler et al. 2017, Eekhout 2010).

For anuran studies there are active and passive sampling methods. Active methods involve visual encounters, diving, netting, nest counts, egg biomass and auditory methods, while passive methods involve artificial hiding places, traps (such as pitfall and funnel trap), PVC pipes and acoustic methods (Eekout 2010). This way, acoustics comprises both active and a passive method of sampling anurans (Eekout 2010, Sugai et al. 2018), representing a very useful way to estimate the species richness of anurans having large numbers for various areas of biological sciences such as integrative taxonomy, applied on ecology, conservation biology and methodological studies (Padial et al. 2010), particularly in relation to habitat use studies, research techniques and species assessment (Dayrat 2005). However, the standardization of field survey methods is still incomplete due to the great diversity of the group, with a wide range of behaviors (Farmer et al. 2009), temporal activity patterns (Farmer et al. 2009), habitats (Rödel & Ernst 2004), and population densities (Tanadini et al. 2011) this way is recommend the use of a combination of methods (Farmer et al. 2009).

Studies using PAM (passive acoustic monitoring) in terrestrial environments began in the 1990s and have grown in recent decades due to its advantages such as the fact that it's a less invasive method, requiring few visits to the study site, causing less interference in the species' vocalization site, in addition to being cost-effective in the long term (Sugai et al. 2018). In this way, using the PAM it's possible to elaborate faunal inventories and conduct monitoring to understand and describe patterns of variation in communication (Bridges & Dorcas 2000), abundance, uniformity, species richness and rotation, variations in daily and seasonal activities (Bridges & Dorcas 2000, Obrist et al. 2010, Melo 2020), along gradients and before climate variations and changes (Llusia et al. 2013). In species inventories, regular acoustic surveys are very useful for determining species composition, but like any methodology there are limitations, so they must be associated with other sampling techniques (see Eekhout 2010). PAM has advantages like the possibility of records multiple taxonomic groups (as birds, anurans, primates, bats, and some arthropods), study nocturnal species and ease of scaling up spatial sampling including at remote localizations (Melo 2020, Melo et al. 2021). At the same time there is some challenges for this technique, as overlapping vocalizations, signals that propagate non-uniformly, and limitations on power and storage, and of course, anthropophony and geophony (Ross et al. 2023). That's why the present proposal which uses the PAM methodology combined with active monitoring (AM). The same proposal was applied by Melo et al. (2021), however, they were restricted to lentic environments (temporary and permanent), at night and in the Cerrado morphoclimatic domain, while this was carried out in lotic environments (waterfall and stream), in the day and night periods of the Atlantic Forest morphoclimatic domain.

Therefore, this study tested whether species richness differs when comparing active and passive monitoring surveys in detecting anurans in lotic environments of Itinguçu State Park. In this way, we detected species richness in 18 days of daytime and nighttime acoustic monitoring (passive and active) and defined six comparative scenarios (with different sampling efforts) to identify the combined effect of both on the efficiency of detection of anuran species. According to what was seen in the literature, it is expected that the PAM detects a greater species richness. This is the first study applying PAM of anurans at lotic environments, and we hope that it helps ecology, conservational and methodological research in tropical environments and in the determination of adequate sampling efforts when applying acoustic monitoring techniques.

MATERIALS AND METHODS

Study area

The study area is located in the Itinguçu State Park (PEIT) which is a full protection conservation unit located in the municipality of Peruíbe, south coast of the state of São Paulo, inserted in the Atlantic Forest morphoclimatic domain with an area of 5,040 hectares (-24º387372”S/-47º017203”W) (Hartung & Campolim 2017). Its vegetation is characterized by dense rainforest, with restinga areas, mangroves, beaches, and rocky shores on its coastal plain, where it’s also possible to observe different lotic and lentic environments, the latter seasonal and permanent. The coastal plain has an altitude of 0 to 20 meters with an average temperature of 24.5ºC. The average annual seasonal rainfall is between 2,800-2,900 mm, with the months of January, February and March having the highest rainfall (Tarifa 2004). During the visits, temperature and humidity data were collected.

Data collect

The data collection period began on April 24, 2022, and ended on February 10, 2023, with the visits taking place in the months of April, June, August, October, December, and February, totalizing six PEIT visits every 45 days as was done by Melo et al. (2021). Three sampling points were designated on the coastal plain of the park. “Point 1 – Arpoador” located on Arpoador beach, and the points 2 (Sítio) and 3 (Guarauzinho). “The point 1 – Arpoador” is located 1,30 km to “point 2 – Sítio”, and the “point 2 – Sítio” is located 0,09 km to “point 3 – Guarauzinho” (Fig. 1). The collection of anurans data took place through two different methods: passive acoustic monitoring (PAM) and active monitoring (AM). For the AM, at least two observers were designated and consisted of visual and acoustic search of adult individuals in the entire perimeter around the place of installation of the recorder and in all directions (Scott & Woodward 1994). This perimeter represents at least 10 or 20 meters of the installation point in all the direction, when possible, because the environment is represented by big trees and rocks, and tall vegetation that can comprises some dangerous animals or fill a hole. The sampling effort was 60 minutes per point according to the number of observers (30 min per observer when two; 20 min when three observers) (Melo et al. 2021). At each of the three points, Sony ICD-PX240 recorders were installed at least one meter above the ground/water depth and protected against the weather and recorded in” wav.” format. Were realized three consecutive days of records each 45 days, totalizing 18 field work. At the end of the fields, the recordings were digitalized, and we selected 5 minutes of recording every hour of the day, totaling 120 minutes of recording/day each. This 5 five minutes of recording were choose following the recommendation of Gooch et al. (2006) that affirms that 5 minutes are sufficient

Figure 1. Sampling sites at Itinguçu State Park, Peruíbe-SP with field activities carried out between April 26, 2022, and February 10, 2023. (a) site 1 "Arpoador" (b), site 2 "Sítio montante" (c) site 3 "Sítio jusante" and (d) represents a recorder installed at one of the sites.

to detect 94% of all anuran species. However the typical length of a survey (generally 5 to 10 minutes depending on the program protocol) has been shown to miss a substantial number of species detections, with more species being detected even after 40 minutes of sampling (Pierce & Gutzwiller 2007).

General recordings (monitoring) will be deposited at Fonoteca Neotropical Jacques Vielliard (FNJV), located at the State University of Campinas (UNICAMP). The research database recordings (selected songs for analysis) were deposited in the Sound Collection (HCLP-S) of the Herpetology Laboratory of UNESP, Campus do Litoral Paulista (LHERP).

All specimens collected were deposited in the Amphibian Collection (HCLP-A) of the Herpetology Laboratory of UNESP, Campus do Litoral Paulista (LHERP). The team for each visit had at least two researchers and a maximum of three. When the visit had only 2 people the sampling time were of 1 hour (30 minutes for each researcher), and when 3 peoples the sampling time were of 1 hour too (20 minutes for each researcher). For the active search methodology, flashlights and manual captures were used. The captured specimens were handled and transported/accommodated to the Núcleo do Arpoador (sorting site) following recommendations in the relevant literature (Campbell & Christian 1982, McDiarmid 1994, Auricchio & Salomão 2002, CONCEA 2018). In addition, the specimens were transported in plastic bags since the containment and handling took place with the hands at the time of collection and euthanasia. The specimens collected were inducted to death according CONCEA (2018), Federal Council of Veterinary Medicine – CFMV (2013), and American Veterinart Medical Associaton – AVMA (2020).

The specimens were fixed in 10% formaldehyde and preserved in 70% alcohol (Campbell & Christian 1982, McDiarmid 1994). Tissue (leg muscle) samples were collected and preserved directly in 100% alcohol and stored in freezers. It should be noted that all campaigns were accompanied by the environmental license of the System of Authorization and Information on Biodiversity (SISBio), and the Instituto Florestal, of the Secretariat of Infrastructure and Environment of the State of São Paulo was contacted for permission to collect specimens in its administration area.

Species richness

To calculate the species richness were choose two indices: one to observed richness represents for Shannon-Wiener, and the Jacknife Index for estimated richnesss. The species recorded per site during each AM survey were used to calculate these two indices, and the species registered in the 5 minutes of recordings were used to calculate these two indices for each site in each hour of the PAM. Clippings were performed using WavePad software and manual visual and auditory inspection of the spectrograms was performed using Raven Pro 1.6 software (Cornell Lab of Ornithology). Also, the observed richness index (Shannon) and the estimated richness index (Jacknife1) of species were applied for each of the methods.

Sampling scenarios

To evaluate the detection efficiency of each method, we defined six sampling scenarios to compare the species richness recorded by AM and PAM. Both methods are characterized by a different temporal distribution of the sampling effort. While the AM is typically an intensive survey that concentrates the sampling effort in short periods, with long intervals between observations (Scott & Woodward 1994), the PAM is often conceived as a regular sampling that records species activity in temporal, fine windows collected at short intervals over the long term (Sugai et al. 2019). To create realistic scenarios that allow us to test the study hypotheses, we developed comparative scenarios grouped into three categories: 1) biased for AM sampling effort (one hour AM to two hours PAM; and 1B: three days AM to one day PAM); 2) equal sampling effort (2A: three days AM day and night; and 2B: eighteen days PAM day and night); and 3) PAM-biased sampling effort (3A: one day AM to three days PAM; and 3B: three days AM to eighteen days PAM). This comparison makes it possible to determine the combined effect of sampling duration and sampling effort on species detection, based on the differences in each method.

Group 1: Biased-towards-AM sampling effort

Scenario 1A: Two hours PAM. The first scenario compares the number of species observed by AM and PAM in two sampling hours (morning/night), corresponding to the same day and time. While the AM allocated 120 minutes of intense *in situ* research (60 min day/60 min night), the PAM recorded only 10 minutes of ambient sounds (5 min day/5 min night) and therefore the two methods used a markedly different sampling effort. The times contemplated for data collection were 10-11 am (day) and 10-11 pm (night).

Scenario 1B: One day PAM. The second scenario compares the number of species observed by each of the methods in a full sampling day. While AM allocated 360 minutes of polling on a given day, PAM logged 120 minutes spread over 5 minutes an hour throughout the day. Thus, the PAM estimate is based on 1 hours since the start of the AM and comprises 24 samples (5 min recordings). The times contemplated for data collection were 10-11 am (day) and 10-11 pm (night).

Group 2: Equal sampling effort

Scenario 2A: Three sampling days AM and PAM. In this scenario, we evaluated species detection with PAM collection observations during three sampling days. To balance the comparison between AM and PAM, we selected audio recordings from three consecutive days since the day the AM was performed and during the same daytime and nighttime hours. This resulted in equal sampling effort for the two techniques with a different temporal distribution of samples. While AM allocated 360 minutes of research on a given day, PAM recorded 360 minutes spread over 3 days (recordings of 5 minutes/hour/day).

Scenario 2B: Eighteen sampling days AM and PAM. We also evaluated the species richness observed by each method during the total number of sampling days. In this case, we compared the species richness estimated by AM (2,160 minutes) with that estimated by PAM (2,160 minutes). This comparison considered both monitoring carried out during the day and those carried out at night, therefore, 24 hours per day of sampling is considered.

Group 3: Biased-towards-PAM sampling effort

Scenario 3A: Three-full-day PAM. In this scenario we compare AM with recordings collected over one full day (120 minutes)., and we selected PAM recordings from three consecutive days (360 minutes). This resulted in a biased sampling effort for PAM that allows us to test the effect of one of the main advantages of PAM compared to AM, namely its ability to cover daily cycles.

Scenario 3B: Eighteen-full-day PAM. Finally, the last scenario explored the maximum sampling capacity of the PAM by comparing the species richness estimated by three days of AM (360 minutes) with those 18 full PAM days (2,160 minutes). This resulted in a highly biased sampling effort for PAM, representing the highest performance of PAM, intensively monitoring daily and seasonal cycles.

Species accumulation curves

To further explore these two methods, we calculated species accumulation curves with incidence-based data, where the sampling unit was the randomly sampled survey time (Chao et al. 2014). We use two scales of sampling units: (i) the number of sampling visits and (ii) the number of sampling days. Thus, two curves (one for each sampling unit scale) were calculated for each sampling technique (i.e., AM and PAM from scenarios 1A to 3B), totaling fourteen species accumulation curves.

Statistical analysis

To compare the detection efficiency of species detection by each method (AM and PAM), the generalized linear model (GLM) was applied to evaluate the residual values (errors) of the models that present a distribution different from the normal one (Gaussian). The analysis was performed in the R Studio program version 4.0.2 (R Development Core Team 2023) and the following packages were used throughout the analysis: “dplyr” (Wickham & Francois 2015), and “ggplot” (Wickham 2016). The data used for analysis were the 18 days of sampling and the abundance detected by each of the methods (AM and PAM) on each of these days, with a Poisson error structure.

RESULTS

Anuran communities

Active monitoring (AM) estimated a total regional richness of 16 anuran species with an average of 8 species per sampling site (7-9; Table I), with four species being observed in all visits as can be seen in the table II. In total, 25,920 minutes of recordings were collected by PAM. There were 2,160 minutes of recordings by PAM (5 min/hour) and 51,840 minutes by AM (120 minutes/day) in 18 days of *in situ* work. In summary, PAM

Table I. **List of species found in the frog community of Itinguçu State Park (Peruíbe-SP), based on active search (AM) and passive acoustic monitoring (PAM) with 18 days of recording (total PAM) and large-scale passive acoustic monitoring, named of scenario PAM 1A (one hour of PAM), PAM 1B (single day of PAM), PAM 2A (3 nights of PAM), PAM 2B (18 nights of PAM), PAM 3A (3 full days of PAM), and PAM 3B (18 full days of PAM).**

Species	AM	PAM 1A	PAM 1B	PAM 2A	PAM 2B	PAM 3A	PAM 3B
Bufonidae							
Rhinella granulosa (Spix, 1824)	X						
Rhinella hoogmoedi Caramaschi and Pombal, 2006	X	-	-	-	-	-	-
Rhinella ornata (Spix, 1824)	X	X	-	-	X	-	-
Craugastoridae							
Haddadus binotatus (Spix, 1824)	X	-	-	-	-	-	-
Cycloramphidae							
Rr Thoropa taophora (Miranda-Ribeiro, 1923)	X	-	-	-	-	-	-
Hylidae							
Aplastodiscus arildae (Cruz and Peixoto, 1987)	X	-	-	-	-	-	-
Boana albomarginata (Spix, 1824)	X	-	-	-	-	-	-
Boana faber (Wied-Neuwied, 1821)	X	-	X	X	X	X	-
Boana semilineata (Spix, 1824)	X	-	-	-	-	-	-
Dendropsophus microps (Peters, 1872)	X	-	-	-	-	-	-
Itapotihyla langsdorffii (Duméril and Bibron, 1841)	X	-	X	X	X	X	-
Ololygon littoralis (Pombal and Gordo, 1991)	X	-	X	-	X	X	-
Scinax granulatus (Peters, 1871)	X	-	-	-	-	-	-
Scinax cf. ruber	X	-	-	-	-	-	-
Hylodidae							
Hylodes dactylocinus Pavan, Narvaes and Rodrigues, 2001	X	-	-	-	-	-	-
Leptodactylidae							
Leptodactylus paranaru Magalhães et al. 2020	X	-	-	-	-	-	-
Odontophrynidae							
Proceratophrys belzebul Dias, Amaro, Carvalho-e-Silva, and Rodrigues, 2013	X	-	-	-	-	-	-
Anuran sp. 1	-	-	X	-	X	X	X
Anuran sp. 2	-	-	X	-	X	X	X
SPECIES RICHNESS	16	1	5	2	6	5	2

detected a regional richness of 7 species at the three fixed sites with an average of 2.3 species per point (0-4). Of all the species detected, only 7 were detected by PAM: *Boana faber*, *Itapotihyla langsdorffii*, *Ololygon littoralis, Rhinella ornata*, and two indeterminate anurans. AM estimated a regional richness of 16 anuran species with an average of 5 species per sampling site (7-9). Only 4 of the species observed by AM were detected by PAM.

Overall, temporal patterns of calling activity were more species-specific on both daily and seasonal scales. In each visit, the species vocalized at a different time, with only two species sharing an acoustic niche: *B. faber* and *I. langsdorffii* at site 3 “Guarauzinho”. The peak of vocalization activity occurred between 7-10 pm (Fig. 2). Only one diurnal species was recorded and awaits identification. On days with torrential rain, no individuals were found

Table II. Compilation of the results of the data collected in each visit.

VISIT	MEAN Temp. (°C)	MEAN HUmid. (%)	Nº OF SPECIES DETECTED (PAM)			Nº OF SPECIES detectED (AM)			SPECIES (PAM)	SPECIES (AM)	TOTAL OF SPECIES BY VISIT
			P1	**P2**	**P3**	**P1**	**P2**	**P3**	**All sites**	**All sites**	
C1 (April)	22°C	80%	0	0	0	1	1	2	-	*L. paranaru*, *P. belzebul*, *R. ornata*, *O. littoralis*	4
C2 (June)	21°C	82%	0	2	1	5	3	4	Anuro sp. 1, *R. ornata*	*Dendropsophus microps*, *L. paranaru*, *R. ornata*, *O. littoralis*, *T. taophora*	8
C3 (August)	20°C	89%	0	-	2	1	3	4	*R. ornata*, *O. littoralis*	*H. binotatus*, *I. langsdorffii*. *P. belzebul*, *R. ornata*, *O. littoralis*, *T. taophora*	6
C4 (October)	23°C	87%	0	2	3	5	4	3	*B. faber*, *I. langsdorffii*, *R. ornata*, *O. littoralis*, Anuro sp. 1. Anuro sp. 2	*B. albomarginata*, *H. binotatus*, *H. dactylocinus*, *I. langsdorffii*, *L. paranaru*, *R. ornata*, *T. taophora*	11
C5 (December)	24°C	88%	0	1	2	2	4	3	*B. faber*, *I. langsdorffii*, Anuro sp. 1, Anuro sp. 2	*I. langsdorffii*, *L. paranaru*, *O. littoralis*, *S. fuscoivarius* *S.* cf. *ruber*, *R. hoodmoedi*, *R. ornata*, *T. taophora*	10
C6 (February)	25°C	87%	0	2	3	2	5	5	*B. faber*, *I. langsdorffii*, *O. littoralis*, Anuro sp. 1	*B. albomarginata*, *B. semilineata*, *H. dactylocinus*, *I. langsdorffii*, *L. paranaru*, *R. ornata*, *O. littoralis*, *T. taophora*	9
TOTAL DETECTIONS PER SAMPLING site			0	7	11	16	20	21	6	16	

at any of the three recording sites through the AM. In addition, the noise caused by the rain influenced the collection of vocalizations by the recorders. Therefore, on days of torrential rain, the presence of individuals was not recorded.

Site 1 "Arpoador" the PAM did not generate any recording of vocalization. This was due to the great noise provided by the waterfall, which is almost five meters away from the place where the recorder was positioned (Fig. 3b). Other locations were considered for the installation of the recorder, however, they were very close to the areas where tourists have access (downstream), and the change in physiognomy of the place was not conducive to the collection of vocalizations (upstream), because the speed and height of the water got bigger. If the individuals were close to the recorder, it would have been possible to perform some recording. On the other hand, through MA, 8 species were recorded at the site: *Aplastodiscus arildae*, *Boana albomarginata*, *Boana faber*, *Haddadus binotatus*, *Leptodactylus paranaru*, *Ololygon littoralis*, *Rhinella ornata*, and *Thoropa taophora*. At this site, all species were observed at night, between 7-9 pm, with June being the month with the highest number of species detected at the site. All detected species were found on the rocks, except for the record of a *R. ornata* which was found inside an earth hole on the left bank ravine, in addition to *T. taophora* being the only species found at this site in all visits.

At site 2 "Sítio" the PAM detected two species (waiting identification), one vocalizing

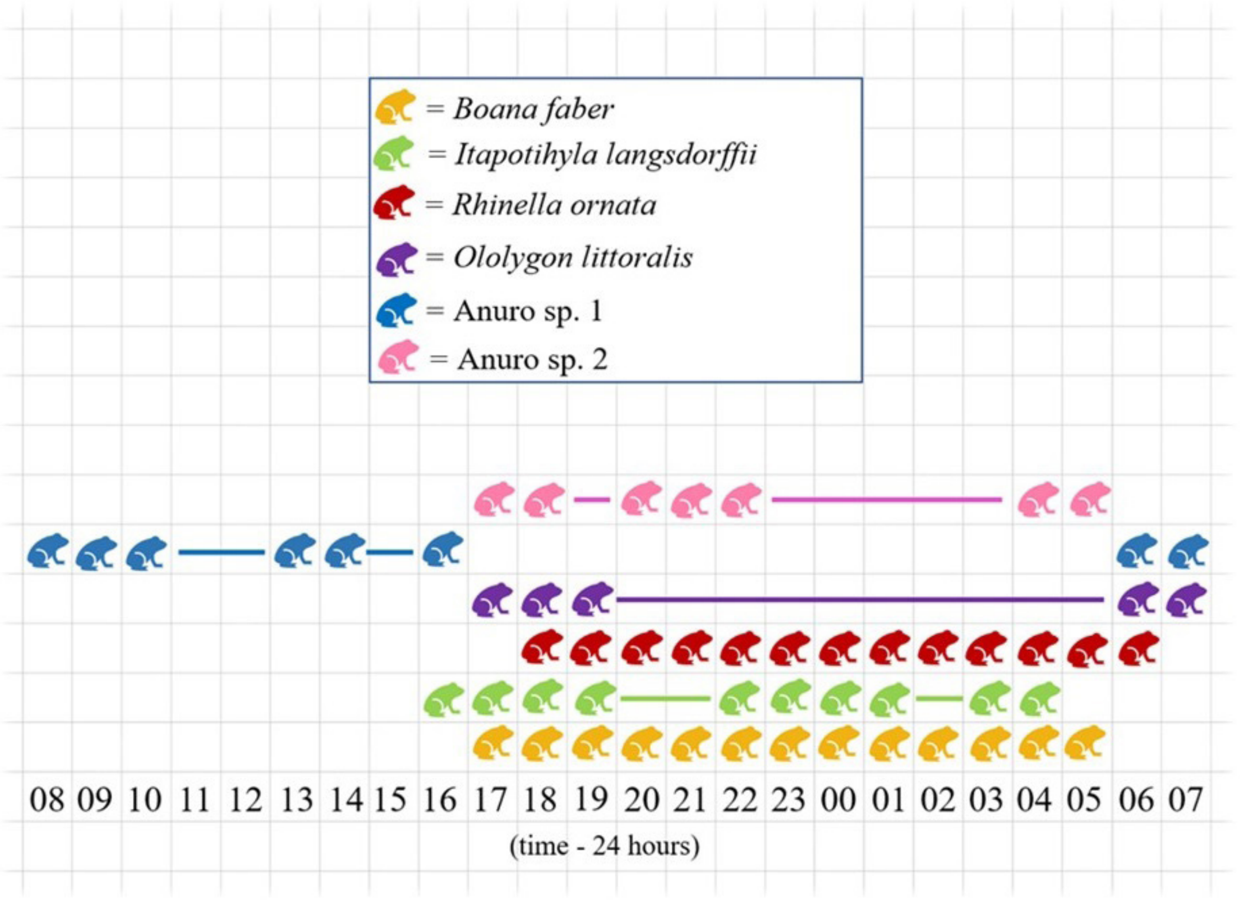

Figure 2. Times of vocalizations recorded by species at Itinguçu State Park, Peruíbe-SP with field activities carried out between April 26, 2022, and February 10, 2023. The 'x' axis shows the time from 8:00 am to 8:00 pm (24-hour period) and the images of frogs indicate the species that vocalized.

during the day and the other during the night. This was the only site that captured diurnal vocalization, as well as the only site where species were sighted by AM also during the day, namely *Hylodes dactynocilus*, *Rhinella ornata* and *Ololygon littoralis*. This was the site with the highest detection of species by the AM, most of them species with arboreal habits. The species recorded at this site were: *Boana albomarginata, Dendropsophus microps, Haddadus binotatus, Hylodes dactynocinus, Itapotihyla langsdorffii, Leptodactylus paranaru, Ololygon littoralis, Rhinella ornata, Scinax* cf. *ruber, Thoropa taophora.*

Finally, at site 3 "Guarauzinho" the PAM detected the highest number of vocalizations, with a total of four species detected: *Boana faber, Itapotihyla langsdorffii, Rhinella ornata* and *O. littoralis*. All vocalizations were obtained at night between 7 pm to 3 am. The species with the highest number of records in different visits were *B. faber* and *I. langsdorffii* (both in October, December, and February). The AM at this site detected nine species: *B. albomarginata, B. semilineata, I. langsdorffii, L. paranaru, O. littoralis, Proceratophrys belzebul, R. hoogmoedi, R. ornata*, and *T. taophora*, with *R. ornata* being the only species found at this site in all visits carried out.

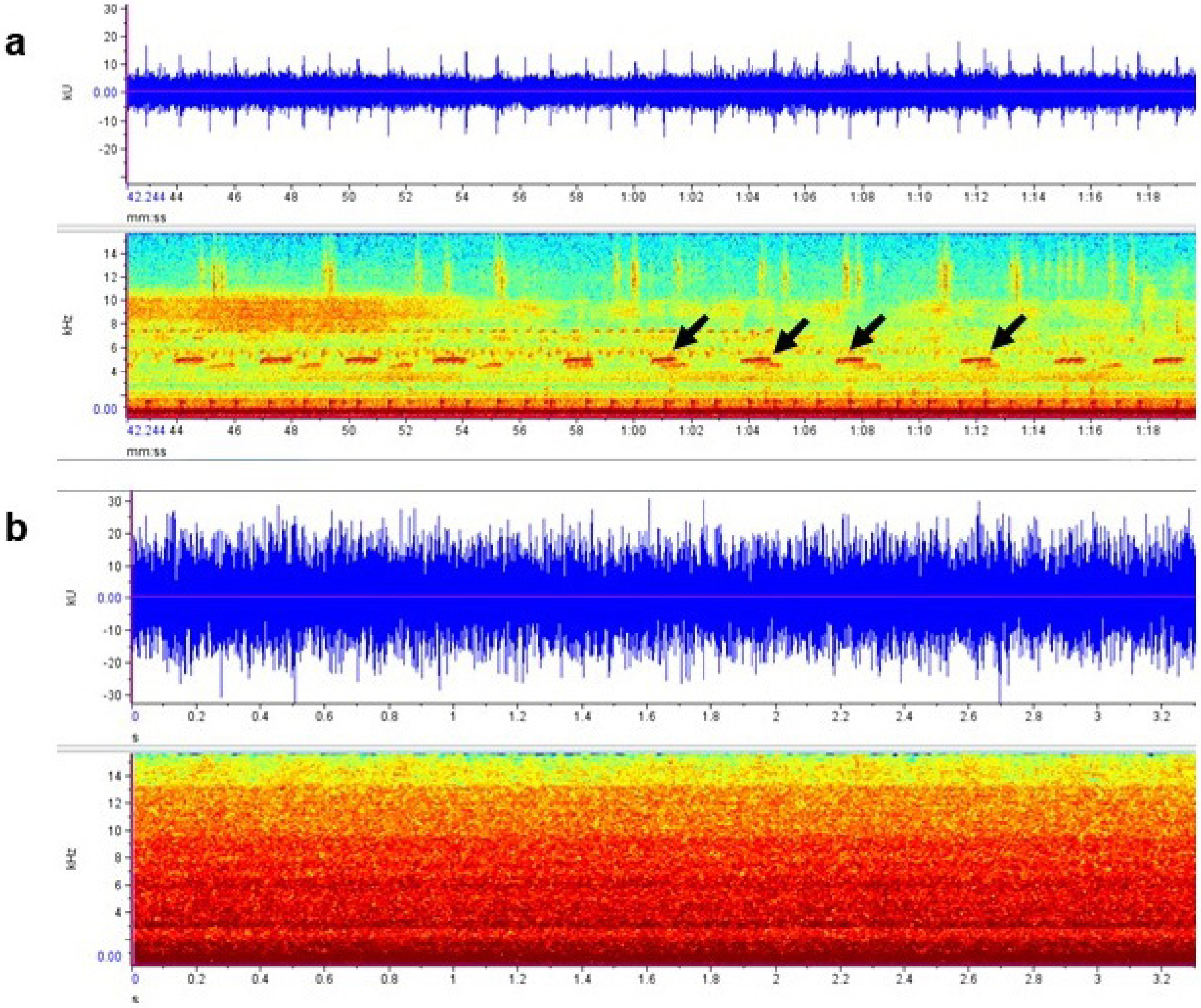

Figure 3. Oscillograms and spectrograms referring to the vocalizations detected by the PAM at Itinguçu State Park, Peruíbe-SP with field activities carried out between April 26, 2022, and February 10, 2023. Advertisement call of *Itapotihyla langsdorffii* recorded at site 3 "Sítio jusante" (a), and representation of noise interference in the recordings at site 1 – "Arpoador" (b). The arrows indicate the detection of the species call among environmental sounds.

After clipping the recordings using the WavePad, they were analyzed using the RavenPro software (Cornell Laboratory Ornithology), where the spectrograms enabled the detection and observation of the species calls. Four spectrograms (and oscillograms) were observed containing the song of the species. Figure 3a represents a recording identifying *I. langsdorffii* with arrows pointing to the detection of the individual's call, and the other represents a recording of site 1 "Arpoador" where the high noise rate is demonstrated for the possibility of detecting individual vocalizers (Fig. 3b). Calls can be identified by small groupings of red "spots" (upper sounds). These were identified and marked while listening to the recording in conjunction with observing the spectrograms. It was observed that each spectrogram had different characteristics, such as background noise (the colder colors, the less noise).

Species accumulation curves

In general, both techniques showed a tendency to increase detections, both AM and PAM (Fig. 4), and in this study we observed a greater efficiency of AM than PAM in detecting species in lentic environments. Species accumulation curves in all scenarios did not show stabilization trends (Figs. 5 and 6). This suggests that additional species remain undetected in most cases. However, in the last two visits, the curves showed stabilization in active monitoring (Fig. 7), however, the general trend is increasing. Thus, the accumulation curves confirmed the tendency towards an increase in species detection by both techniques, thus revealing an increase in species richness, with greater sampling effort by both methods. In small temporal scale scenarios, AM proved to be more efficient in species detection than PAM. However, the efficiency of PAM showed an increase in a longer time scale. In general, the AM proved to be more efficient than the PAM for a survey of anuran species in lotic environments.

Sampling scenarios

On a shorter time scale, the record of anuran species by PAM was significantly lower than that recorded by AM in each of the visits carried out (Fig. 5b), as expected. The trend of scenarios 1A and 1B tends towards an increase in detection, but with R² 0.3383 and 0.2571 respectively, which suggests a trend with low significance. In a scenario with equal sampling effort (Fig. 5c), AM proved to be more efficient than PAM, with an increase in species detection with each visit carried out (4-8 per visit) than PAM (0-4 per campaign) , however, it is observable that species detection by PAM in scenario 2B (18 days of PAM) tends to increase more than scenario 2A, which demonstrates that species detections increase in relation to the increase in temporal scale. In the sampling effort in scenarios 3A and 3B, there was greater detection of species per visit by PAM (0-8), but AM still proved to be more efficient (4-8) (Fig. 5d). However, we can highlight that it was during the visit carried out in June that PAM (in scenario 3B) surpassed AM in the number of detections (AM=5; PAM=7), which was

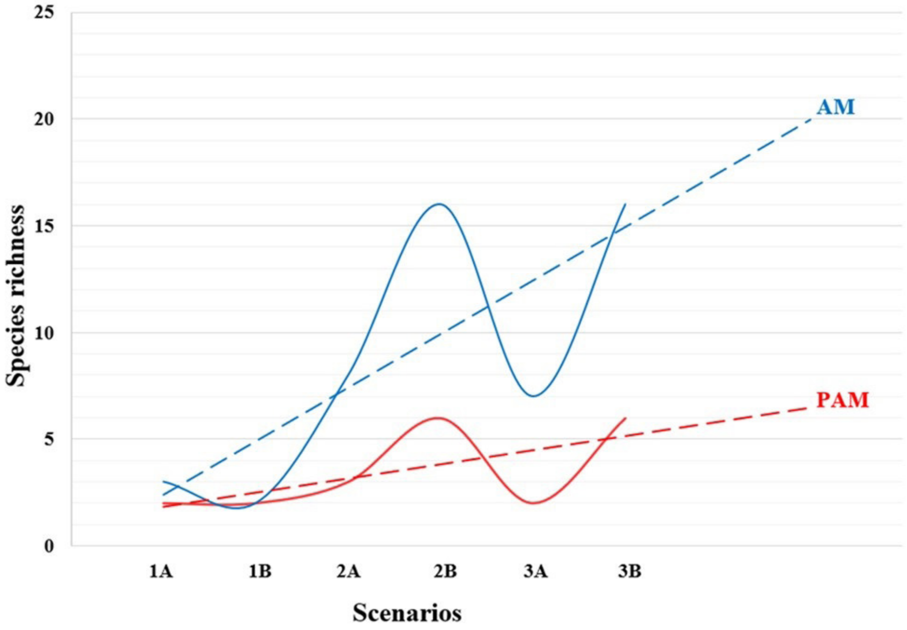

Figure 4. Linear trend of the two methods in relation to scenarios (x axis) and species richness (y axis). The solid blue line represents the detection of species by AM and the solid red line represents the detection of species by PAM. The blue dashed line represents the AM trend line and the red dashed line that of the PAM. AM, R²=0.5905. PAM, R²=0.3875.

not expected, as we hypothesized that June would be a month with low anuran activity.

In the present study, the predominance of AM efficiency ($R^2=0.9605$) in relation to PAM ($R^2=0.3988$) was generally observed in a lotic environment, which can occur due to several factors to be discussed below. However, the tendency of both applied methods is to increase in temporal scale and by temporal sampling effort (Figs. 5 and 6). Regarding the analysis of generalized linear models (GML), the data used were the 18 days of sampling and the abundance detected by each of the methods (AM and PAM) on each of these days. This analysis resulted in an $R^2=0.224$ between methods (Fig. 8). The analysis corroborated the linear trend graphs with the AM detecting a greater abundance than the PAM, however, the difference in the efficiency of both methods was small, as expected after the analysis of figure 4.

DISCUSSION

Our six sampling scenarios revealed that the detection of anuran species in lotic environments by AM was more efficient than PAM overall (AM=16; PAM=6), but it should be considered that the PAM may have been impaired by environmental noises that manage to camouflage the vocalizations depending on the distance the individual is from the recorder (Fig. 3). It's possible to observe that with the increase in the sampling effort, the PAM tends to present a greater detection of species (Fig. 6)

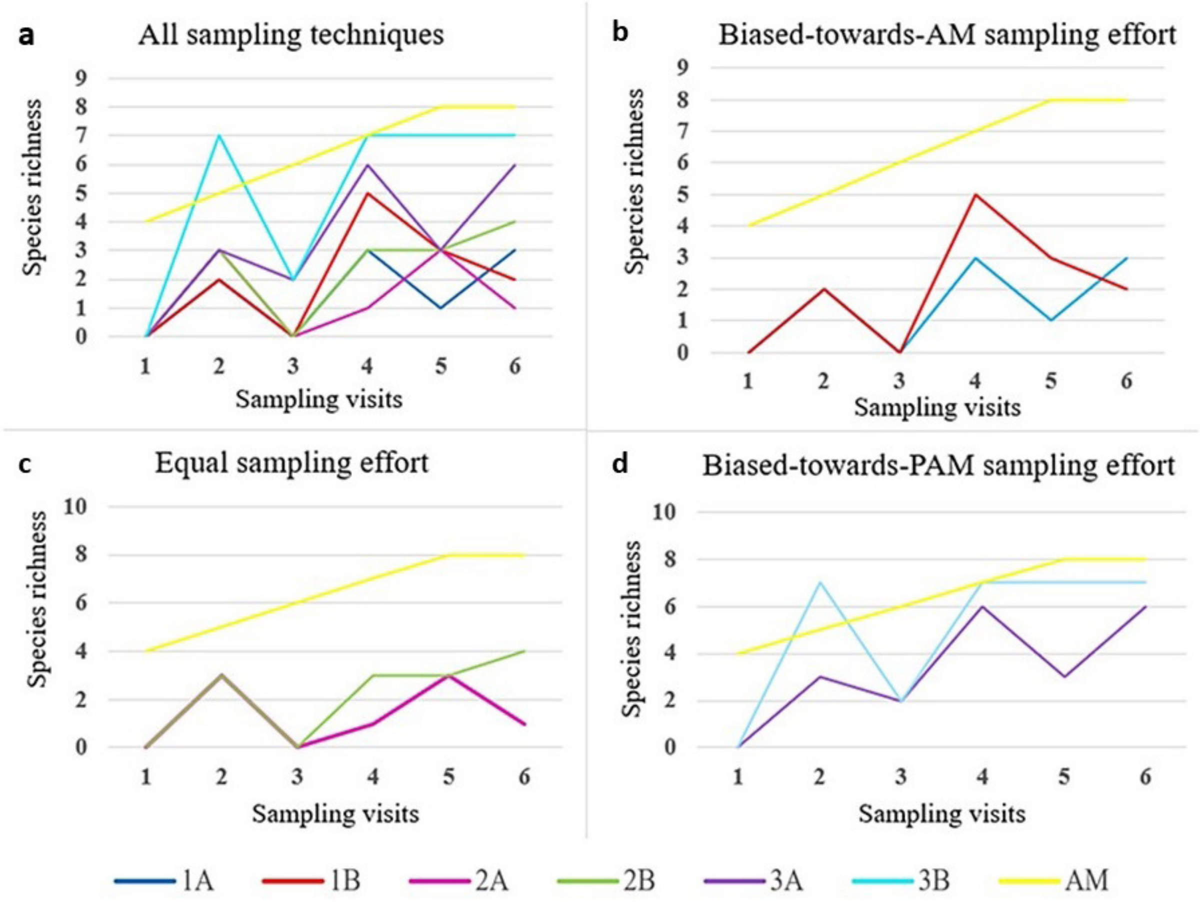

Figure 5. Species richness accumulation curves observed at each visit in each of the techniques and scenarios.

(Gotelli & Colwell 2011). The literature presents PAM as a very efficient method for detecting anuran species (Parris et al. 1999, Hsu et al. 2005, Acevedo & Villanueva-Rivera 2006, Madazzolo et al. 2017, Melo et al. 2021) due to the possibility of a continuous survey and without human interference at the vocalization site (Peterson & Dorcas 1994, Bridges & Dorcas 2000, Köhler et al. 2017), however these studies are usually carried out in lentic environments such as puddles, marshes and ponds with hydroperiod varying between permanent and temporary. Melo et al. (2021) was the main study that guided the present one, but the authors' tests were applied in lentic environments, during the night and in the Cerrado morphoclimatic domain. Now, the present was applied in a lotic environment, with records being collected day and night, in the Atlantic Forest morphoclimatic domain. Thus, it's not surprising that our results are contrasting. While Melo et al. (2021) recorded 12 anuran species by AM and 21 by PAM, the present recorded 16 species by AM and 6 by PAM. This difference may be due to differences between environments (lentic/lotic), sampling effort (night/24 hours), type of automatic recorder (programmable/non-programmable), and morphoclimatic domais (Cerrado/Atlantic Forest). Most anurans usually use lentic environments to reproduce (Wells 2007), therefore it is more likely to detect a greater species richness, in calling activity, in lentic environments than in lotic ones, once that AM detect species that are not in their reproduction site, as in the case of *Haddadus binotatus*. They are animals whose majority tend to have nocturnal habits (Wells 2007), however some species are diurnal, so it is more likely to find them during the night than during the day, which was demonstrated here in which only one frog was detected by PAM during the day and three detected by AM (Fig. 2). In addition, each morphoclimatic domain has characteristic differences between biotic and abiotic factors, which influence ecological interactions (intra and interspecific), influencing regional richness (Wells 2007).

Although AM has been shown to be more efficient in detecting anuran fauna than PAM, it is notable that the efficiency of PAM increases in time scale, which can be seen in figure 1 and

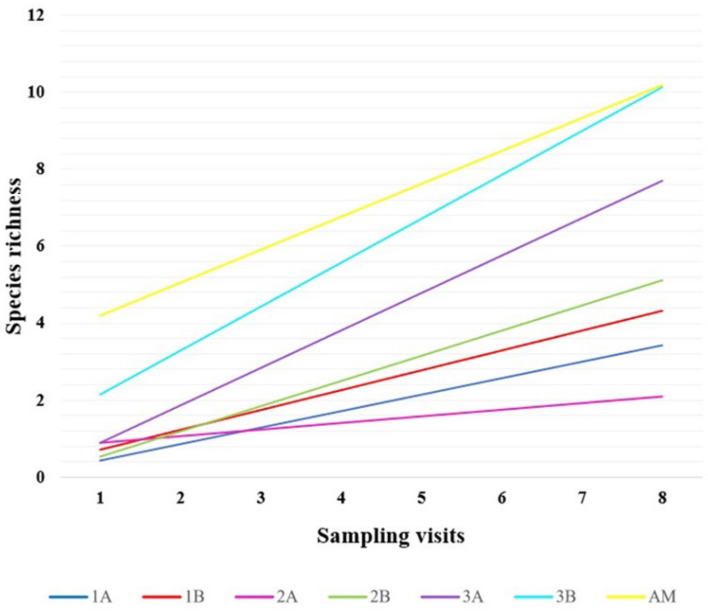

Figure 6. Trendlines from all sampling scenarios and from active monitoring (AM).

stated in the literature (Sugai et al. 2018). The trend lines point to the AM with the highest trend in species richness, followed by scenarios 2A and 2B, which reaffirms the increase in the efficiency of the PAM on a temporal scale. However, we must consider that the visits began in April (dry winter) and ended in February (rainy summer), which already points to a trend towards an increase in species due to the increase in temperature and rainfall. It should be noted that two of the six species detected by PAM were not detected by AM, as well as the recording of an anuran vocalizing during the day that does not correspond to any of the species observed by during the day (*H. dactylocinus, O. littoralis* and *R. ornata*). This demonstrates the efficiency of both techniques when combined, totaling a regional richness of 18 species, corroborating the literature (Acevedo & Villanueva-Rivera 2006, Dorcas et al. 2010, Silva 2010). In addition, our monitoring included data collection (active and passive) morning and afternoon, which is not commonly done (Sugai et al. 2018). Through the PAM it was observed that the patterns of vocalization activity of the species show the times (on a 24-hour scale) at which times the species were recorded vocalizing, perceiving a peak of vocalization activity between 7-10 pm (Fig. 3), as well as in Narvaes et al. (2009) and Melo et al. (2021).

The recordings obtained were cut and analyzed, which resulted in spectrograms and oscillograms. The use of spectrograms and oscillograms for detection and analysis of the acoustic parameters of the species calls is essential in PAM (Köhler et al. 2017). Unfortunately, the recordings from site 1 "Arpoador" were not able to detect the calls of anurans due to environmental noise (Fig. 3b), however, in the other points the detection was "clean", and the calls were easy to visualize in the spectrogram (Fig. 3a), as presents by Ross et al. (2023). At site

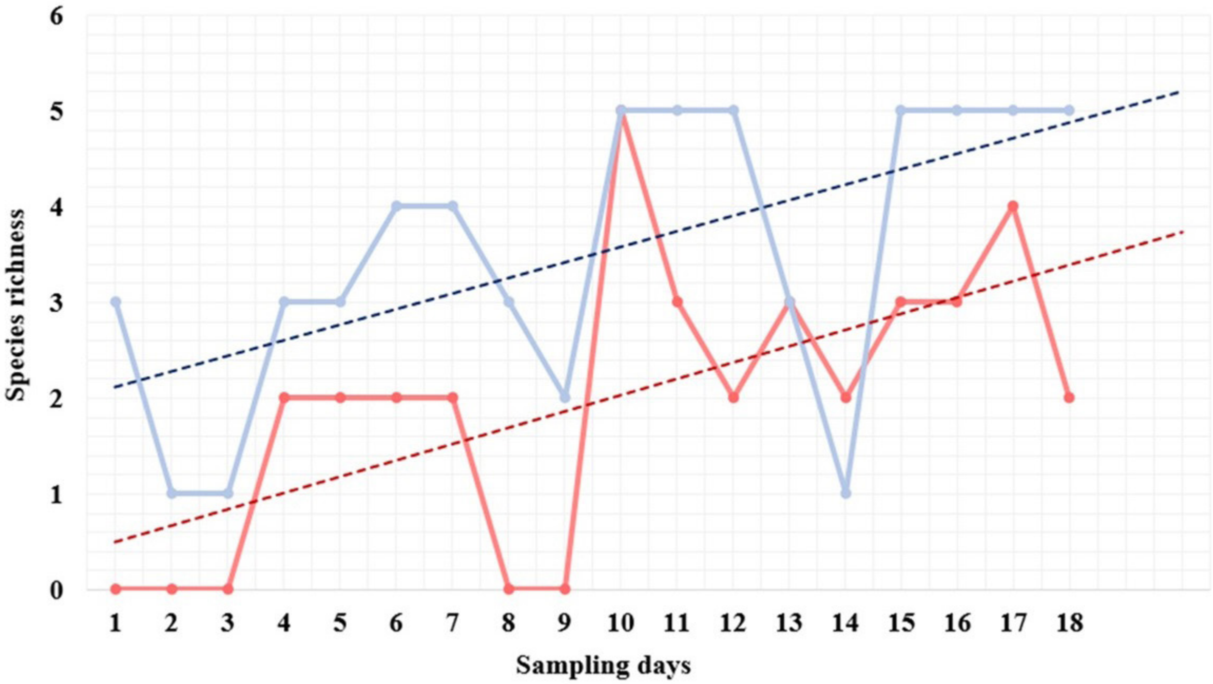

Figure 7. **Species accumulation trend curve for both techniques (AM in blue; PAM in red) for each monitoring day. The dotted lines represent the trend lines for each of the techniques. This curve has a low p-value ($p < 0.003554$) with T-test = -2.99802.**

1 “Arpoador” it was not possible to collect any data through the PAM, while the AM registered eight species. This is due to background noise in which all environmental sounds end up masking vocalizations, such as the sound of water flowing and crickets very close to the recorders. The interference of environmental noise masking the vocalization of anurans has already been detected by Xie et al. (2015) who suggest a methodology for extracting this type of noise. However, it was observed that although the AM had recorded eight species at this point, *A. arildae* was in the process of metamorphosis, probably stage 42 (Gosner 1960), individuals of *L. paranaru* were found spawning, and none of the individuals of *R. ornata* and *T. taophora* did not have a vocal sac and did not sing (advertisement and distress), which characterizes them as females (Moura et al. 2021). Already, *O. littoralis* was observed vocalizing.

At site 2 “Sítio” the PAM detected an anuran vocalizing during the day. We believe it is a hylodid, as the species of this family have diurnal habits (Sá 2013) and when we were exploring the area downstream, we found tadpoles of *Phantasmarana* cf. *curutuensis*, which represents a species of hylodid. On the other hand, the AM registered three species during the day, *H. dactylocinus, R. ornata* and *O. littoralis*, which were also registered in the active nocturnal monitoring. At this site, not many species were found close to the recorder (only *B. albomarginata*, *H. binotatus* and *O. littoralis* in the vegetation), the other species were on rocks around the stream in the upstream direction.

Site 3 “Guarauzinho” was the site with the highest detection of species by PAM (4) and by AM (9), totaling 10 species at this point. We believe that a probable hypothesis for recording a greater number of species in this area was because the forest is closed, there is more vegetation over the water, creeping and erect herbaceous plants, bushes, and trees, one of the margins has a flat profile (while the other has a flat profile). flat and ravine profile) (Vasconcelos & Rossa-Feres 2005). At this site the speed of the water flow increases more than at site 2 “Sítio”, as well as an increase in the water depth,

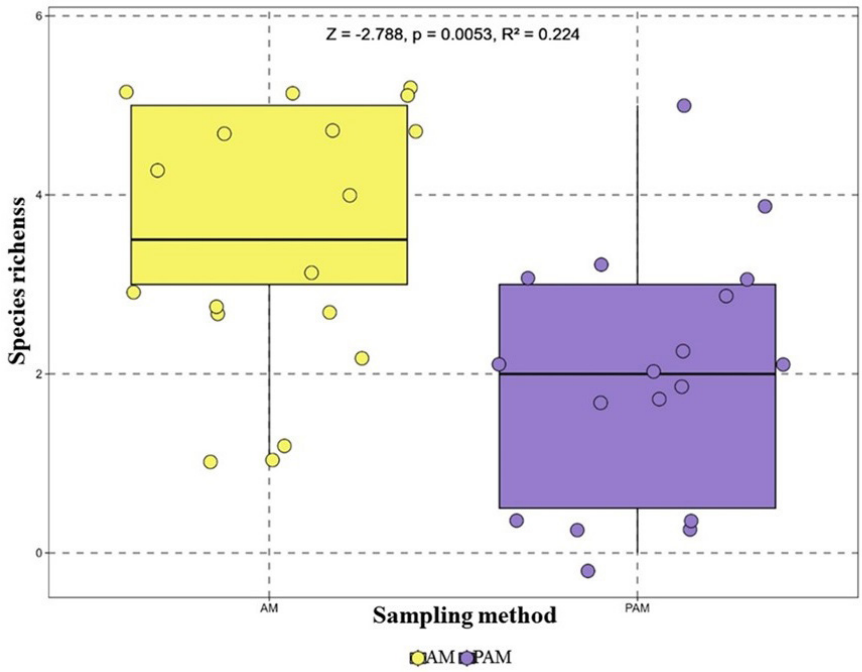

Figure 8. **Result of the GLM analysis in the detection of abundance in each of the methods used (AM and PAM). The circles represent the residual values of each method, the yellow ones referring to AM and the purple ones referring to PAM. The Z value represents that the residual values are below the mean. The p value < 0.05, which does not make the probability of observing a greater difference less significant. The R² represents the fit to the regression, showing how well the regression predictions approximate the true values.**

however, we believe that the fact that there is a swamp about 40-50 meters from the point where the recorder was installed interferes with this diversity, as spawning females were also recorded at this site. Acoustic niche partitioning was detected at point 3 “Guarauzinho” with *I. langsdorffii* and *B. faber* vocalizing at the same time. At the other points, acoustic niche partitioning was not detected.

As expected, the AM and PAM trend curves (Fig. 4) tended to increase, as well as all PAM scenarios (Figs. 5 and 6) showed a tendency to increase species detection with increasing sampling effort, which is expected for the morphoclimatic domain, suggesting that additional species have not yet been recorded. In scenario 1, in which AM was compared to PAM, on a time and day scale, biased towards AM, AM proved to be much more efficient, as expected (Fig. 5b). The accumulation curve and trend line can be seen in figure 4, with the relationship of species richness *versus* visits. In scenario 2, in which AM and PAM were compared with the same sampling effort (3 days of monitoring/18 days of monitoring, both 24 hours *per* day), AM also proved to be more efficient than PAM. With scenario 2A representing the lowest efficiency and trend (Fig. 5c and Fig. 6, pink line), while scenario 2B, which included data collection at the same times as scenario 2A, but over 18 days of monitoring, presented a greater detection efficiency, which was expected, the increase in species detection with increasing sampling effort.

In the last scenario that was biased towards PAM, the results showed that this was the scenario with the highest detections by PAM, in both scenarios 3A (3 full days) and 3B (18 full days). During all the comparisons between the scenarios and the AM, the AM always proved to be more efficient, however, in scenario 3 it is possible to observe in figure 5 that the estimation of detections of the scenario 3B (light blue) reaches the same level as the AM according to a greater number of visits, which reinforces the high efficiency of PAM in longer time scales that is observed in several studies (Acevedo & Villanueva-Rivera 2006, Madalozzo et al. 2017, Melo et al. 2021). The scenarios that demonstrated greater detection of species by PAM are those that have greater temporal effort (Fig. 5d). In addition, the GLM analysis corroborated the linear trend graphs with the AM detecting a greater abundance than the PAM, as expected after the analysis of figure 8.

Although AM had a better performance than PAM in this study, we emphasize that due to the high plasticity of characteristics of the life history of frogs, individuals may probably not be present during the sampling times of a given day or season (Parris et al. 1999, Gooch et al. 2006, Willacy et al. 2015). Previous studies have tested the efficiency of AM and demonstrated that this technique can be replaced or combined with PAM. Both techniques for monitoring anuran populations are usable and provide us with good results, although they are better when used in combination, since both have limitations (Acevedo & Villanueva-Rivera 2006, Dorcas et al. 2010, Silva 2010). For example, while AM interferes with the vocalization site and can drive individuals away from the area, PAM, in turn, does not detect the presence of females, being restricted to only vocalizing male adults (Dorcas et al. 2010). The combination of AM and PAM methods for the detection of adult anurans is commonly used, mainly due to its low cost and relatively short duration (Dorcas et al. 2010, Madalozzo et al. 2017). Furthermore, we point out that the efficiency of any sampling technique also depends on the environmental heterogeneity, the biotic and abiotic conditions of the habitats, cost, time invested, personal requirements and effectiveness itself (Corn et al.

2000, Rocha et al. 2004, Hsu et al. 2005, Dorcas et al. 2010).

Our results demonstrated that in lotic environments the active monitoring method was more efficient than the passive acoustic monitoring in all sampling scales (scenarios). However, when both techniques are united more species tend to be detected. Although the PAM was not as efficient in a low temporal scale, it tends to increase in efficiency with longer sampling duration. Thus, for long-term studies, it is strongly suggested that PAM be used in conjunction with AM. Although PAM is mostly used and efficient in lentic environments, despite its limitations, such as, for example, the impossibility of directional recording for better results, when combined with AM, it represents a complementary data source that helps to understand patterns of species activities. Thus, the present work suggests that for studies of anuran surveys in lotic environments, AM should be used in a short-term study, however, for long-term studies, the data would be much more complete by combining AM with PAM.

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