EVALUATION OF SURVEY METHODS FOR SAMPLING ANURAN SPECIES RICHNESS IN THE NEOTROPICS

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ABSTRACT. Species richness is central to ecological theory, with practical applications in conservation, environmental management and monitoring. Several techniques are available for measuring species richness and composition of amphibians in breeding pools, but the relative efficacy of these methods for sampling high-diversity Neotropical amphibian fauna is poorly understood. I evaluated seven studies from south and south-eastern Brazil to compare the relative and combined effectiveness of two methods for measuring species richness at anuran breeding pools: acoustic surveys with visual encounter of adults and dipnet surveys of larvae. I also compared the relative efficacy of each survey method in detecting species with different reproductive modes. Results showed that both survey methods underestimated the number of species when used separately; however, a close approximation of the actual number of species in each breeding pool was obtained when the methods were combined. There was no difference between survey methods in detecting species with different reproductive modes. These results indicate that researchers should employ multiple survey methods that target both adult and larval life history stages in order to accurately assess anuran species richness at breeding pools in the Neotropics.

KEYWORDS. Amphibian; Breeding sites; Generalized linear mixed model; Monitoring; Sampling techniques; Species inventory.

INTRODUCTION

Species richness is a central theme of ecological theory (Magurran, 2004). Currently, the importance of species richness in ecological studies is underscored by its frequent use as a key response variable in ecological studies and frequent application for purposes of conservation, environmental management and monitoring (Bunge and Fitzpatrick, 1993; Colwell and Conddington, 1994; Gotelli and Colwell, 2001; Magurran, 2004; Chao et al., 2005; Chao et al., 2006). Because species richness is such an important and widely used biological variable, accurate estimates are imperative. Methods for measuring species richness typically vary with the biology of the taxon of interest; however, empirical studies comparing the effectiveness of different survey methods, and thus the accuracy of species richness estimates, are lacking for many taxa in most regions of the world. Such information might also be used to establish protocols for bio-inventories, which may be important in regions with high biodiversity and/or limited resources.

Several techniques are available for measuring species richness and composition of amphibians and the techniques to be used in a particular study will be guided by the question being asked by the investigator (Heyer *et al.*, 1994). Ecologists and conservation biologists interested in amphibian distribution frequently wish to understand which factors influence species richness or occurrence of amphibians in

breeding sites (Beja and Alcazar, 2003; Van Buskirk, 2005; Werner *et al.*, 2007). This becomes especially important when we take into account phenomena such as worldwide amphibian declines, which seem to have affected even populations in pristine habitats (Alford and Richards, 1999; Houlahan *et al.*, 2000; Kiesecker, Blaustein and Belden, 2001). To study the drivers of anuran species richness and distribution, researchers survey breeding pools using standard methods for monitoring amphibian assemblages (Heyer *et al.*, 1994; Lips *et al.*, 2001). The most common methods used to sample anurans in breeding pools are acoustic survey with visual encounter of adults (*sensu* Scott and Woodward, 1994) and survey of larvae with dipnetting (Shaffer *et al.*, 1994).

Most studies of anuran species richness rarely span more than a few years for a given breeding pool, and sampling frequency for longer-term studies is generally low within each reproductive season. As a result of limited survey effort, some species often remain unsampled. Anuran behavior also limits survey efficiency, as most anuran adults spend a short time (less than a week) at breeding pools and many "explosive-breeding" species are present in breeding pools for periods as short as a few days (reviewed in Wells, 1977). Efficiency of dip-net surveys of anuran larvae is affected by the physical structure of breeding pools. For example, larvae of some species take shelter in the vegetation or deep-water microhabitats and thus are very difficult to access by dip-net. Several methods have been proposed for sampling amphibian populations (Heyer *et al.*, 1994; Lips *et al.*, 2001), but the relative efficacy of these methods for sampling highly diverse neotropical amphibian fauna has not been investigated (Doan, 2003). Here I use data from seven studies in south and southeastern Brazil, which used the same survey methods, to evaluate how accurately the different methods estimated species richness of anurans at breeding pools. Specifically, I compare the efficacy of acoustic surveys with visual encounter of adults (SAVE hereafter) and survey of larvae with dipnetting (SLD hereafter), and I also evaluate the efficiency of the two methods (SAVE + SLD) combined.

MATERIAL AND METHODS

I gathered data on anuran surveys from seven studies (Table 1) that used the same sampling methods to estimate the anuran species richness in breeding pools at different localities in south and southeastern Brazil (Fig. 1). The studies are located in a small latitudinal range (from $20^{\circ}12 \square S$ to $26^{\circ}51 \square S$) and their maximum altitudes range from 420 m to 1500 m above sea level.

Acoustic surveys consisted of recording the presence of calling male anurans of each species using species-specific calls. Visual encounter surveys consisted of field personnel performing a systematic, time-constrained walk through a breeding pool searching for animals (see Heyer *et al.*, 1994). SLD is the simplest method for sampling larvae in temporary and permanent ponds and/or stream habitats with limited access or great structural complexity. SLD surveys consisted of field personnel performing equal numbers of dip-net sweeps, evenly distributed throughout each breeding pool (Shaffer *et al.*, 1994). The collection was made with a long, wire, hand net (3 mm² mesh size). The effort was standardized by

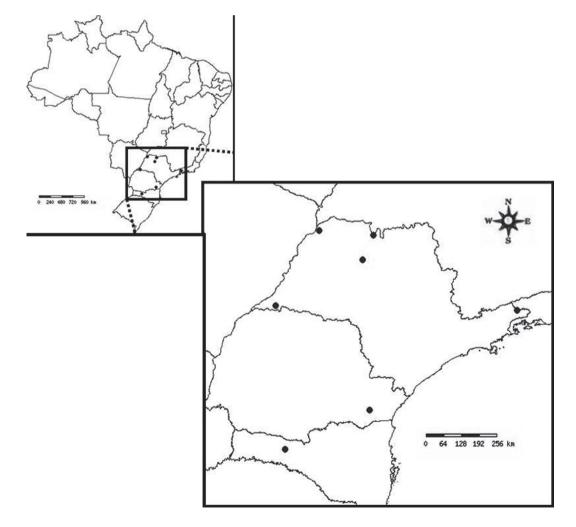


FIGURE 1. Distribution of the seven studies in south and southeastern Brazil whose data were used in the analysis.

Sites	Longitude	Latitude	Altitude	PS	SF (days)	References
Icém	-49.198	-20.340	423	September 2004 to March 2005	19	Candeira 2007
Nova Itapirema	-49.533	-21.100	478	January 2003 to March 2004	25	Vasconcelos and Rossa-Feres 2005
Santa Fé do Sul	-50.926	-20.211	430	September 2003 to August 2004	18	Santos et al. 2007
Araucária National Park	-52.031	-26.856	1100	January 2007 to January 2008	13	Conte 2010
Gralha Azul Experimental Farm	-49.308	-25.658	920	January 2002 to March 2003	15	Conte and Rossa-Feres 2007
Morro do Diabo State Park	-52.333	-22.500	599	February 2006 to March 2007	12	Vasconcelos 2009
Serra da Bocaina National Park	-44.587	-22.642	1500	July 2008 to June 2009	12	M. V. Garey and D. B. Provete (unpubl. data)

TABLE 1. Description of seven studies in south and southeastern Brazil whose data were used in the analysis. PS = Period of sampling; SF = sampling effort in each breeding pool.

passing the net along the banks of ponds and streams, intending to sample all the available microhabitats. The seven studies used standard techniques for inventory and monitoring of adults and anuran larvae, SAVE and SLD, respectively, in at least four breeding pools in each locality. The search for anurans was made along the perimeters of breeding pools using visual encounters and also by listening for males engaged in calling activities. Tadpole sampling was carried out in the same breeding pools where the adults were monitored.

STATISTICAL ANALYSIS

To compare species richness recorded in all breeding pools with an equal sampling effort, species accumulation curves were generated using the samplebased rarefaction method in the program EstimateS 8.20 (Colwell 2004). Number of taxa was plotted as a function of accumulated number of samples in 500 randomizations. The function of expected richness (Sobs) is the accumulation function of species sampled. The expected richness function is called Mao Tau and allows confidence intervals to be calculated for the direct statistical comparison of species richness among survey methods (Colwell 2004). The data necessary to conduct this analysis were available only for three of the seven studies (Candeira 2007, Conte and Rossa-Feres 2007, Santos et al. 2007), therefore I also analyzed all seven datasets using a generalized linear mixed-model approach (GLMM, Venables and Ripley, 2002).

To test whether each sampling method produced the same count of species present in each breeding pool, I performed GLMM with a Poisson error distribution and log link function in R software version 2.10.1 (R Development Core Team, 2005). I performed GLMM because my response variable (number of species detected in each breeding pool) is a discrete count and many authors recommend that count data should not be analyzed by log-transformation (O'Hara and Kotze 2010, Zuur et al. 2010). GLMM were developed using the lmer function in the lme4 package of R (R Development Core Team, 2005). The lmer function has the facility to deal with complicated error structures and hence avoid the pitfalls of pseudoreplication (Crawley 2007). I considered species richness and survey method (SAVE, SLD and sum of both techniques) as fixed effects, with breeding pools as a random effect. I used contrast coefficients to test the hypotheses that SAVE + SLD is different from the sum of both techniques and to test whether SAVE is different from SLD (Crawley 2007). The idea was to establish contrasts, or specified comparisons between particular sets of means that test specific hypotheses (Gotelli and Ellison 2004). I performed analyzes without considering sampling effort, because in each study, sampling effort for SAVE and SLD were the same in each breeding pool (Table 1). Therefore, as I wished to test whether estimated species richness at each breeding pool was the same for each survey method, sampling effort was not considered in the model.

To determine whether SAVE and SLD differ in detecting species with different reproductive modes, I classified reproductive modes of anuran species recorded in the seven studies according to the classification criteria of Haddad and Prado (2005; Table 2). Then, for each of the seven datasets, I used Chi-square contingency analysis to test whether survey methods (SAVE or SLD) differed in the success at which they detected species with different reproductive modes.

RESULTS

Species accumulation curves showed that both methods SAVE and SLD recorded a lower number of species for the localities of study than the two techniques combined (Fig. 2), but the difference was statistically significant only for Santa Fé do Sul (Fig. 2). SAVE was a better method than SLD in Gralha Azul Experimental Farm, but it was worse than SLD in Santa Fé do Sul (Fig. 2). There was no difference between SAVE and SLD in Icém (Fig. 2). When all breeding pools from all seven studies were considered separately, the combined SAVE/SLD method detected more species than either technique (SAVE or SLD) did individually for 38 of 55 breeding pools (approximately 1/3; Fig. 3, Table 3). Using only a single survey method reduced the species richness at all seven localities, except Araucária National Park where only four breeding pools were sampled, relative to the estimate using the combined SAVE/ SLD technique (Fig. 3, Table 3). The number of species detected using each sampling method at each breeding locality (Fig. 3) varied greatly, and many species were detected using only one of the techniques (Fig. 3).

In the seven studies evaluated, ten reproductive modes were recorded in total (Table 1, Fig. 4). There was no variation in reproductive modes recorded between SAVE and SLD (Fig. 4): Icém ($\chi^2 = 0.18$, df = 3, P = 0.98), Morro do Diabo State Park ($\chi^2 = 0.35$, df = 5, P = 0.99), Nova Itapirema ($\chi^2 = 2.34$, df = 3, P = 0.5), Santa Fé do Sul ($\chi^2 = 0.52$, df = 3, P = 0.91), Serra da Bocaína National Park ($\chi^2 = 0.33$, df = 6, P = 0.99), Gralha Azul Experimental Farm ($\chi^2 = 1.66$, df = 7, P = 0.97) and Araucária National State Park ($\chi^2 = 0.39$, df = 7, P = 0.99).

DISCUSSION

Ecologists who conduct field surveys of species richness have long recognized that it is virtually impossible to detect all species and their relative abundances with a limited number or intensity of samples (Chao *et al.*, 2005). Results showed that when survey methods for anurans in breeding pools (SAVE and SLD) are considered separately, total species richness is severely underestimated. However, when the SAVE and SLD methods are used together, survey results closely match the actual number of species. Some sampling bias will be present regardless of survey methods used, but when the methods are combined the number of species recorded in each breeding pool is probably a sufficient approximation of reality. All sampling methods have some advantages and disadvantages in estimating anuran species richness. Some species breed so explosively that they are rarely detected via acoustic surveys, but these species can be better surveyed using the SLD method because the

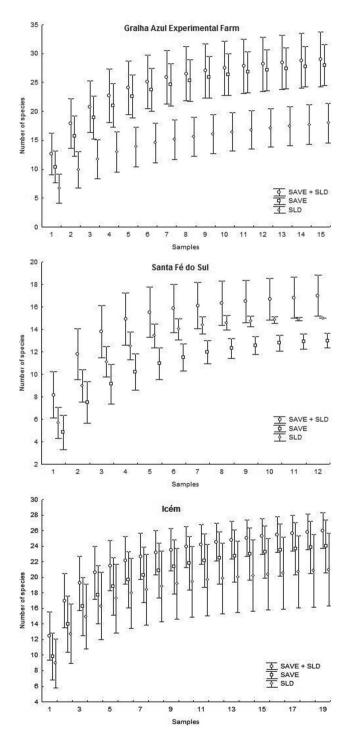


FIGURE 2. Species accumulation curves of sample-based rarefaction (Mao Tau) with confidence intervals generated by 500 randomizations for different sampling methods. SAVE = acoustic survey and visual encounter of adults; SLD = survey of larvae with dipnetting and SAVE + SLD = sum of both techniques.

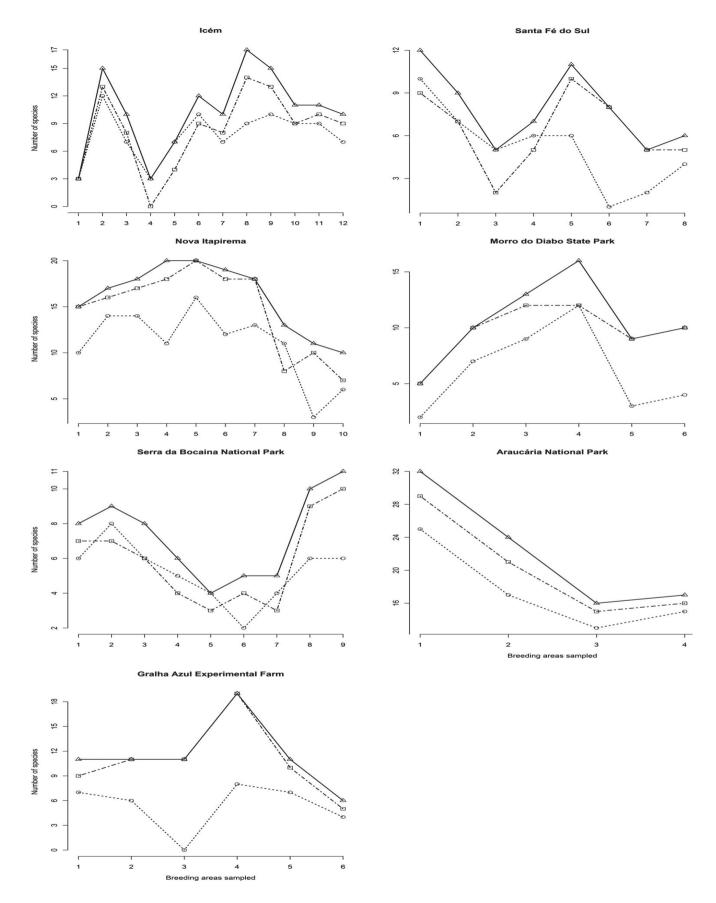


FIGURE 3. Number of anuran species detected using acoustic survey and visual encounter of adults (square and dashed lines), survey of larvae with dipnetting (circle and dashed lines) and sum of both surveys techniques (triangle and solid lines) in different breeding pools in seven localities in south and southeastern Brazil.

TABLE 2. Diversity of reproductive modes in anurans observed for the seven studies in south and southeastern Brazil whose data were used in the analysis. (adapted from Haddad and Prado, 2005).

I. Eggs aquatic

A. Eggs deposited in water.

1. Eggs and exotrophic tadpoles in lentic water.

2. Eggs and exotrophic tadpoles in lotic water.

4. Eggs and early larval stages in natural or constructed basins; subsequent to flooding, exotrophic tadpoles in ponds or streams.

5. Eggs and early larval stages in subterranean constructed nests; subsequent to flooding, exotrophic tadpoles in ponds or streams.

- B. Eggs in foam nest.
 - 11. Foam nest floating on pond; exotrophic tadpoles in ponds.
 - 13. Foam nest floating on water accumulated in constructed basins; exotrophic tadpoles in ponds.
- C. Eggs arboreal.
 - 24. Eggs hatch into exotrophic tadpoles that drop in lentic water.
 - 25. Eggs hatch into exotrophic tadpoles that drop in lotic water.
- D. Eggs in foam nest.

30. Foam nest with eggs and early larval stages in subterranean constructed nests; subsequent to flooding, exotrophic tadpoles in ponds.

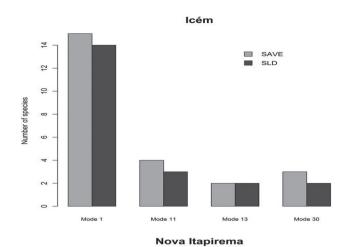
32. Foam nest in subterranean constructed nests; endotrophic tadpoles complete development in nest.

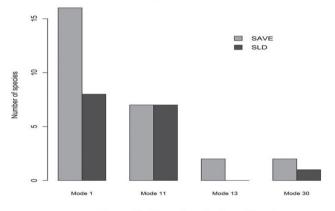
larval phase lasts longer in comparison to the breeding period (Heyer *et al.*, 1994). On the other hand, some larvae are hard to capture by SLD because they can hide on the pool bottom, in vegetation, or use other parts of the breeding site that are difficult to access, so the species are best surveyed using the SAVE technique. Santos, Rossa-Feres and Casatti (2007) in the municipality of Santa Fé do Sul, recorded two explosive breeders, *Physalaemus centralis* Bokermann, 1962 and *Physalaemus marmoratus* (Reinhardt and Lutken, 1862), only by the SLD method, whereas *Leptodactylus chaquensis* Cei, 1950 and *Dendropsophus minutus* (Peters, 1872) were recorded only using the SAVE method. Therefore, knowledge of the constraints and biases of sampling methods is critical for designing appropriate research studies investigating anuran species richness.

	Coefficient	SE	Ζ	р
ICEM				
SAVE + SLD vs SBT	-0.08	0.03	-2.18	0.02
SAVE vs SLD	0.03	0.07	0.5	0.61
NOVA ITAPIREMA				
SAVE + SLD vs SBT	-0.29	0.12	-2.29	0.02
SAVE vs SLD	0.09	0.11	0.79	0.42
SANTA FÉ DO SUL				
SAVE + SLD vs SBT	-0.01	0.05	-1.95	0.05
SAVE vs SLD	0.1	0.1	1.03	0.29
ARAUCÁRIA NATIONAL PARK				
SAVE + SLD vs SBT	-0.05	0.04	-1.24	0.21
SAVE vs SLD	0.07	0.08	0.89	0.37
GRALHA AZUL EXPERIMENTAL FARM				
SAVE + SLD vs SBT	-0.13	0.05	-2.55	0.01
SAVE vs SLD	0.35	0.1	3.27	0.001
MORRO DO DIABO STATE PARK				
SAVE + SLD vs SBT	-0.1	0.05	-1.87	0.06
SAVE vs SLD	0.22	0.1	2.13	0.03
SERRA DA BOCAINA NATIONAL PARK				
SAVE + SLD vs SBT	-0.09	0.05	-1.75	0.07
SAVE vs SLD	0.06	0.1	0.59	0.55

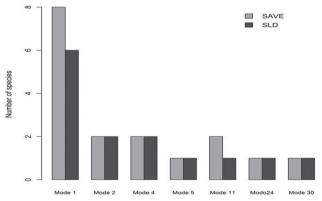
TABLE 3. Summary of the generalized linear mixed model* (GLMM) showing the contrast coefficients between sampling methods. SAVE = acoustic survey and visual encounter of adults; SLD = survey of larvae with dipnetting and SBT = sum of both techniques.

*Model: number of species ~ sampling methods + (1 | breeding areas)





Serra da Bocaína National Park



Gralha Azul Experimental Farm

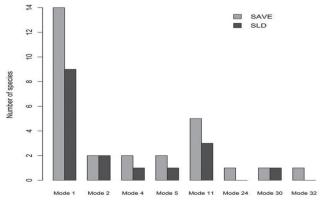
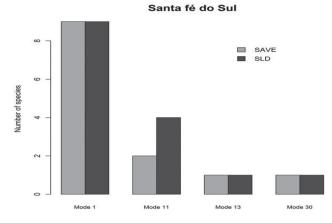
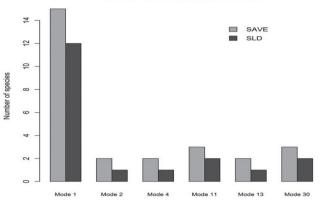


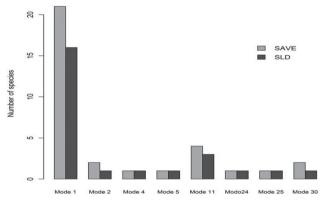
FIGURE 4. Number of reproductive modes in anurans detected using acoustic survey and visual encounter of adults (SAVE – light gray bar), survey of larvae with dipnetting (SLD – dark gray bar) in seven localities in south and southeastern Brazil.



Morro do Diabo State Park



Araucária National State Park



Time, funding, and number of field personnel available to work are other factors of importance during the selection of survey methods (Heyer et al., 1994). Few conservation projects in the tropics have access to the expertise, money, or time necessary for a thorough ecological assessment (Pearman, 1995). SAVE and SLD are considered highly effective and inexpensive techniques for surveying anuran species (Heyer et al., 1994; Scott and Woodward, 1994; Corn, Muths and Iko 2000; Crouch and Paton, 2002). As area, time, and number of species increase, no single method is likely to meet all objectives and a combination of methods will be necessary (Corn, Muths and Iko 2000). Complementarity methods are even more important for detecting species in tropical areas where amphibian communities are far more complex due to higher species richness and taxonomic diversity. Moreover, employing multiple techniques will increase efficiency because researchers will be able to detect more species in a shorter period of time, which reduces costs and frees resources for sampling additional sites.

Neotropical forests harbor the highest global anuran species richness and diversity of reproductive modes (AmphibiaWeb, 2010, Haddad and Prado 2005). Making informed choices about the appropriate sampling techniques requires knowledge of how different methods vary in successfully detecting species with different reproductive modes. Anuran species recorded in this meta-analysis were limited to reproductive modes associated with breeding pools, and no species had direct development or reproductive modes associated with treeholes and bromeliads. Both the SAVE and SLD techniques showed the same efficacy in recording anuran species with reproductive modes associated with breeding pools. The fact that many anuran species have the same reproductive mode facilitates the recording of species with similar reproductive modes by different sampling techniques. Therefore, even though some species are not recorded by a particular sampling technique, species with similar reproductive modes will likely be documented by the same survey method.

It is extremely important to know which survey methods are most appropriate for meeting one's objectives (Doan, 2003). The present analysis identifies significant methodological biases of common techniques for surveying anuran species richness at breeding pools in the Neotropics. Of the two methods discussed in this paper, SAVE was the technique that detected the highest number of anurans in the most breeding pools. However, the composition of species detected by SLD was different from SAVE in the same breeding pool. Therefore, the use of both methods is strongly recommended in future research aiming to assess anuran species richness and composition in neotropical breeding pools.

RESUMO

Riqueza de espécies é um tema central na ecologia teórica com aplicabilidade prática para propósitos de conservação, manejo ambiental e monitoramento. Diversas técnicas são conhecidas para estimar a riqueza e a composição de espécies de anfíbios em um determinado sítio, mas a eficiência dessas metodologias para amostragem da fauna de anuros Neotropicais em poças de reprodução com alta diversidade de espécies permanece escassa. Eu avaliei sete estudos, realizados no sul e sudoeste do Brasil para comparar a eficiência de dois métodos para amostrar riqueza de espécies de anuros em poças de reprodução: levatamento acústico e encontro visual de adultos e levantamento de girinos com puçá. Eu também comparei a eficiência relativa de cada método de amostragem em detectar espécies com modos reprodutivos diferentes. Resultados mostraram que ambos os métodos sub-estimam o número de espécies quando usados separadamente; contudo, quando os métodos são realizados conjuntamente nós melhoramos a eficiência em detectar um número de espécies, sendo o resultado mais próximo do número real de espécies utilizando cada poça de reprodução. Não houve diferença entre os métodos de amostragem no registro de espécies com diferentes modos reprodutivos. Esses resultados indicam que pesquisadores deveriam empregar multiplos métodos que registrem tanto adultos como girinos visando registrar acuradamente a riqueza de espécies de anuros em poças de reprodução Neotropicais.

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