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Original Article

Estimation of a closed population size of tadpoles in temporary pond

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(With 4 figures)

Abstract

The practice of capture-recapture to estimate the diversity is well known to many animal groups, however this practice in the larval phase of anuran amphibians is incipient. We aimed at evaluating the Lincoln estimator, Venn diagram and Bayes theorem in the inference of population size of a larval phase anurocenose from lotic environment. The adherence of results was evaluated using the Kolmogorov-Smirnov test. The marking of tadpoles for later recapture and methods measurement was made with eosin methylene blue. When comparing the results of Lincoln-Petersen estimator corresponding to the Venn diagram and Bayes theorem, we detected percentage differences per sampling, i.e., the proportion of sampled anuran genera is kept among the three methods, although the values are numerically different. By submitting these results to the Kolmogorov-Smirnov test we have found no significant differences. Therefore, no matter the estimator, the measured value is adherent and estimates the total population. Together with the marking methodology, which did not change the behavior of tadpoles, the present study helps to fill the need of more studies on larval phase of amphibians in Brazil, especially in semi-arid northeast.

Keywords: Amphibia, Anura, capture-recapture, diversity, lentic environments.

Estimativa do tamanho da população fechada de girinos em lagoas temporárias

Resumo

A prática de captura-recaptura para a estimação da diversidade é bem conhecida para diversos grupos animais, porém na fase larvar de anfíbios anuros essa prática é incipiente. Objetivamos avaliar os métodos do estimador de Lincoln, diagrama de Venn e o teorema de Bayes na inferência do tamanho populacional de uma anurocenose em fase larvar de ambiente lótico. A aderência dos resultados foi avaliada através do teste de Kolmogorov-Smirnov. A marcação dos girinos para posterior recaptura e aferição dos métodos foi feita com eosina de azul de metileno. Ao compararmos os resultados do estimador de Lincoln que corresponde com o do diagrama de Venn e com o teorema de Bayes detectamos diferenças percentuais por amostragem, isto é, a manutenção da proporção dos gêneros de anuros amostrados é mantida entre os três métodos, embora com valores numericamente diferentes. Ao submetermos esses resultados ao teste Kolmogorov-Smirnov não encontramos diferenças significativas. Logo, qualquer que seja o estimador o valor aferido é aderente e estima a população total. Aliado à metodologia de marcação que não alterou o comportamento dos girinos, o presente estudo ajuda a preencher a necessidade de mais estudos na fase larvar dos anfíbios no Brasil, em especial no semiárido nordestino.

Palavras-chave: Amphibia, Anura, captura-recaptura, diversidade, ambiente lêntico.

1. Introduction

During the decades of 40 and 50 of XX century, the theoretical development for population estimation by capture-recapture of animals was intensified (White et al., 1982). Three approaches explain the main works with population estimators, namely: closed population with simple sampling and the classical sequential capture-recapture, such as the Lincoln estimator (Abuabara and Petrere Junior, 1997; Engen, 1978; Silveira Neto et al., 1976; Otis et al., 1978); Venn diagram (Coeli et al., 2000; Cormack, 1989, 1992; Rivest and Daigle, 2004) and the

Bayes theorem (Castledine, 1981; King and Brooks, 2001; Leite et al., 2000).

In ecology, the first researcher to employ estimation method was Carl GJ Petersen (1896) by studying the fish migration flow in the Baltic Sea. Frederick Lincoln (1930) estimated the population of wild ducks in North America. There were several published studies that used the capture-recapture with anurans (Hero, 1989; Waichman, 1992; Williamson and Bull, 1996), but studies with tadpoles are still incipient.

Capture-recapture methods are used in various areas, from epidemiology (Coeli et al., 2000) to zoological studies of populations (Fernandez, 1995) or even in the analysis of the variation of measurement intervals of closed communities of diverse magnitudes (Cormack, 1992). The capture-recapture techniques are used for closed populations, where the effects of birth rate or mortality and migration are not taken into account, in this case it is considered no change in population size. They can also be used for open populations, where population changes caused by birth rate, mortality and migration are taken into account (Cormack, 1989).

Tadpoles are especially notable for presenting a wide morphological diversity associated with 46 developmental stages (Altig and McDiarmid, 2007; Gosner, 1960) which requires special study conditions, since the development stage and probability of metamorphosis should be taken into account. Lima and Pederassi (2012) and Lima et al. (2014) showed studies in which the stage progress decreases the probability of mortality, but reduces the population size of the species.

In this work we make the case study of a biocenosis of tadpoles in temporary pond through the mark-recapture method and estimate the closed population size, seeking to establish a method to quantify and understand the sharing of tadpole populations.

2. Material and Methods

2.1. Area of study

The hydroelectric dam of Boa Esperança is located 80 km from the city of Guadalupe, State of Piauí, Northeast Brazil with drainage area of 87,500 m^2 with spillways with maximum flow of 12,000 m^3/second. On the banks of the dam there are spillways that generate sporadic water flow making temporary ponds during the period of floods occurring between November and March with rainfall peak equivalent to 356 mm of rain in January.

2.2. Sampling area and unit

Temporary pond formed by the spillway water of the dam is located between the coordinates (S 06° 44’ 52.8”; W 43° 34’ 39.4”). For 15 minutes three researchers used plastic sieve with 15 cm handle, measuring 280 mm × 160 mm × 70 mm with uninterrupted collection and deposition of the tadpoles in a 5 liter plastic container with 160 mm of diameter. After the collection period we waited the stabilization of the sediments and repeated the same methodology.

2.3. Capture and recapture

The collected tadpoles were subjected to eosin methylene blue diluted with water at a ratio of 7.5/1,000, remaining in this solution for 10 minutes, and then they were counted, identified to genus level and released in the same pond. Subsequently, when the pond turbidity was normalized, the procedure was repeated considering the recaptured tadpoles, i.e., colored and new individuals.

There was no change in the animals’ behavior during and after the process of marking the tadpoles with eosin methylene blue.

Lincoln Estimator is given by the Equation 1 below:

$$\frac{m_2}{n_2} = \frac{n_1}{N} \therefore N = \frac{n_1\, n_2}{m_2} \tag{1}$$

N is the population size; n_1 is the total number of elements captured in the first sample; n_2 is the total number of elements captured in the second sample; and m_2 is the number of elements captured in the first and second sample (Abuabara and Petrere Junior, 1997; Silveira Neto et al., 1976).

For increased accuracy of the Lincoln-Petersen’s estimator it was used the Bailey model (Abuabara and Petrere Junior, 1997; Badii et al., 2012; Bailey, 1951; Fernandez, 1995). Where were used the Equation 2 below:

$$N = \frac{n1(n2+1)}{m2+1} \tag{2}$$

The standard error of the estimate is given by the Equation 3:

$$SE_N = \sqrt{\frac{n1^2\,(n2+1)(n2-m2)}{(m2+1)^2\,(m2+2)}} \tag{3}$$

The detection of differences between two estimates of population size is measured by the Equation 4 below:

$$t_{calc} = \sqrt{\frac{N_1 - N_2}{(SE_1)^2 + (SE_2)^2}} \tag{4}$$

t_{calc}= Student’s t test
N_1 = estimate of the population size 1
N_2 = estimate of the population size 2
SE_1 = standard error of the estimate of population size 1
SE_2 = standard error of the estimate of population size 2

If t_{calc} is less than $t_{tabulated}$ for degrees of freedom = α (t_{tab} = 1.96), it is accepted the null hypothesis (the two populations compared do not differ in size). If t_{calc} is greater than the t_{tab} for the degrees of freedom = α, the null hypothesis is rejected and is assumed that the two compared populations present different sizes (Brower and Zar, 1954).

To satisfy the result of the N_P the confidence index (CI) was calculated (Seber, 1982):

a. when the number of individuals marked + number of individuals caught in the second sample was $\geq N_P$;
b. When the number of individuals marked in the second sample was > 7.

Axiom for the CI:

a. When the result of dividing the number of individuals scored in the second sample by the number of individuals caught in the second sample corresponds to > 0.10 to < 50 should be used the Poisson distribution, and > 50 should be used normal approximation to obtain the CI;
b. If the result of the division is < 0.10 should be used the binomial distribution.

The Equation 5 is used for Normal Approximation:

$$\frac{n^2}{m^2} = \pm\left\{ Z\propto \left| \left[\sqrt[2]{\frac{(1-f)\left(1-\frac{n^2}{m^2}\right)\left(\frac{n^2}{m^2}\right)}{(c-1)}} \right] + \frac{1}{2C} \right\} \quad (5)$$

f = Population fraction
1 – f = Correction for finite population
1/2C = Correction for continuity
Zα = normalized value to the confidence level of 1 - α.

Venn Diagram (Coeli et al., 2000; Ferreira et al., 2012; Machado et al., 2013 – see Figure 1)

$PA = \frac{n_a}{N}$: probability of being captured in the first sample

$PB = \frac{n_b}{N}$: probability of being captured in the second sample

$PAB = \frac{n_c}{N}$: probability of being captured in both samples

Bayes Theorem (Gelman, 2008; Gotelli and Ellison, 2011; Paula et al., 2009)

A genus of tadpole can be collected by three researchers 1, 2 and 3. Considering that the three researchers had the same accuracy of collection, i.e., 50% efficiency, hypothetical value, and that previous collections corresponded to: $\%sp_1$; b: $\%sp_2$; c: $\%sp_3$ . . . n: $\%sp_{ni}$ all occupying the same pond. What is the probability of randomly draw out from the pond a particular genus?

Consider the following events:

G = {presence of tadpole}, P1 = {Researcher 1}, P2 = {Researcher 2} and P3 = {Researcher 3}.

We have, therefore, to calculate using the Equation 6 as below:

$$P\{G\} = P\{G/P1\}.P\{P1\} + P\{G/P2\}.P\{P2\} + P\{G/P3\}.P\{P3\}. \quad (6)$$

We know that P{P1%; P2%; P3% ...PN_i%} when we apply the Bayes theorem:

Be $A_1, A_2, A_3, \ldots, A_n$, n mutually exclusive events, as shown in Equation 7 below, we have:

$$P\{A_i\} = \frac{P\{A_i\}.P\{B/A_i\}}{P\{A_1\}.P\{B/A_1\} + P\{A_2\}.P\{B/A_2\} + \ldots + P\{A_n\}.P\{B/A_n\}} \quad (7)$$

Replacing B by genus and A_i by researcher, we ask the question:

Considering that P1, P2 and P3 constitute a partition of the sample space, what is the possibility of obtaining the four genera in a sample?

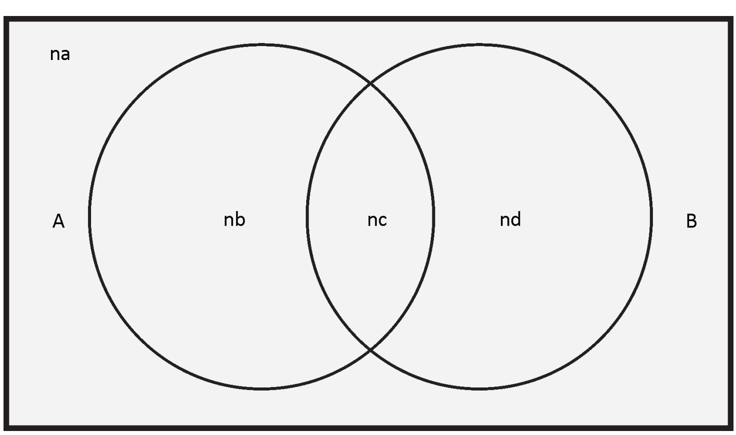

Figure 1. Schematization of the Venn diagram. Sample N sizes = $n_a + n_b + n_c + n_d$; sample A = $(N_A = n_b + n_c)$; sample B = $(N_{B=}\, n_d + n_c)$. n_a = number of non-captured tadpoles in any sample; n_b = number of captured tadpoles in the first sample; n_c = number of captured tadpoles in the first and second sample; n_d = number of captured tadpoles in the second sample.

2.4. Area and volume of the sample space

The area and volume of the pond were obtained through the half ellipse, in which the area is equal to the greatest length (C); greatest width (L) and greatest depth (p): A= (C x L x p)/2 and the volume is equal to $V=4/3p((C/2+L/2+p)/3)^3/2$.

2.5. Occupation and density

The frequency of dominance and relative dominance correspond to the number of specimens of a species by the total number of specimens of the total sampled. The density was calculated using the absolute population by the area.

2.6. Diversity index (Zar, 2010)

Brillouin Index is obtained by the Equation 8 below:

$$HB = \frac{\ln N! - \sum ln_i!}{N} \quad (8)$$

N = Total number of specimens of the total sampled
n = Number of specimens of a species.

The approximation of the factorial of a number was calculated by spreadsheet according to Lima and Batista (2010).

The adherence of the estimators was evaluated using the Kolmogorov-Smirnov test.

3. Results

Three hundred tadpoles distributed in four genera were collected: *Scinax*, *Leptodactylus*, *Pithecopus* and *Pseudopaludicola*. When we submitted the data of the first and second counting of tadpoles to the Lincoln-Petersen estimator, we concluded that the total population corresponded to 1,130 tadpoles, being 51.56% of *Scinax* sp., 18.78% of *Leptodactylus* sp., 3.13% of *Pithecopus* sp. and 26.51% of *Pseudopaludicola* sp., corresponding to the distribution density of 130.18 tadpoles per square meter. The Standard Error (SE) was 62.19 and 60.95, being the differences lower than the tabulated α (0.94). The confidence index (Normal Approach) at 95% was 16.15 (inferior limit), and 39.12 (superior limit).

By using the descriptors of the Venn diagram (see Figure 2) we identified the intersection of 173 tadpoles, being 473 tadpoles considered to the first sample and 525 tadpoles to the second sample. When we estimate the probability of tadpoles by genus and sample, we have in the first sample 33.95% of *Scinax* sp., 9.77% of *Leptodactylus* sp., 1.51% of *Pithecopus* sp., and 12.71% of *Pseudopaludicola* sp.; in the second sample 20% of *Scinax* sp., 2.66% of *Leptodactylus* sp., 0.44% of *Pithecopus* sp., and 3.55% of *Pseudopaludicola* sp. As intersection, i.e., estimated as present in both samples, we have 12% of *Scinax* sp., 1.77% of *Leptodactylus* sp., 0.17% of *Pithecophus* sp., and 1.42% of *Pseudopaludicola* sp. (see Figure 3).

Considering that the three researchers had the same accuracy of collection, i.e., 50% efficiency (hypothetical value), and that previous collections corresponded to: 33% in *Scinax* sp. (S); 9.7% in *Leptodactylus* sp. (L); 1.5% in *Pithecopus* sp. (PH), and 12.7% in *Pseudopaludicola* sp. (PS) all occupying the same pond. What is the probability of randomly draw out from the pond a specimen of a particular genus?

Consider the following events:

G = {presence of tadpole}, P1 = {Researcher 1}, P2 = {Researcher 2} and P3 = {Researcher 3}.

We have, therefore, to calculate using the Equation 6: P{G}=P{G/P1}.P{P1}+P{G/P2}.P{F2}+P{G/P3}.P{P3}.

We know that P{P1} = 1/2; P{P2} = 1/2, and P{P3} = 1/2 and we also know that P{G/S} = 0.33; P{G/L} = 0.09; P{G/PH} = 0.015 and P{G/PS} = 0.12. Applying these values to the expression above (Equation 6), we find:

P{G} = (0.33).(0.5) + (0.09).(0.5) + (0.015).(0.5) + (0.12).(0.5) = 0.165 + 0.045 + 0.0075 + 0.06 = 0.27, i.e., 27%.

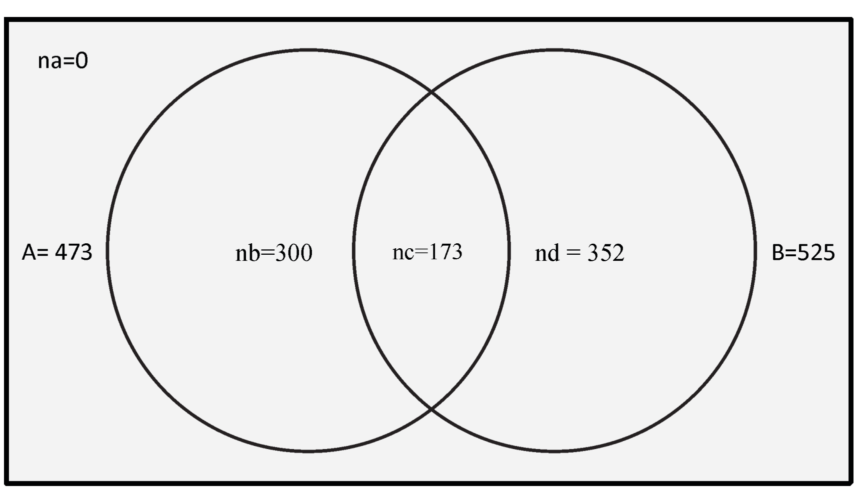

Figure 2. Venn diagram; (A) First Sample; (B) Second Sample, nb tadpoles captured in the first sample, nc tadpoles coincident in both samples, nd tadpoles captured in the second sample.

In a sample the probability of presence of each of the four genera is: *Scinax* sp. = 70%; *Leptodactylus* sp. = 27%; *Pseudopaludicola* sp. = 2.7%; *Pithecopus* sp. = 0%

By submitting the results to the Kolmogorov-Smirnov test no significant differences were found (see Table 1), what shows that no matter the proposed estimator, i.e., Lincoln-Petersen, Venn or Bayes, the measured value is adherent and estimates the total population.

When comparing the results of Lincoln-Petersen estimator corresponding to the Venn diagram and Bayes theorem, we detected percentage differences per sampling (see Figure 4), i.e., the proportion of the genera is kept among the three methods, however the values are different.

The Brillouin diversity index was 0.136123.

4. Discussion

In our case, the community is closed for having as a physical limiter the own pond with an area of 8.68 m^2 and 9,487 ml of water. Studies on fish in streams have been developed demonstrating that it is possible to estimate

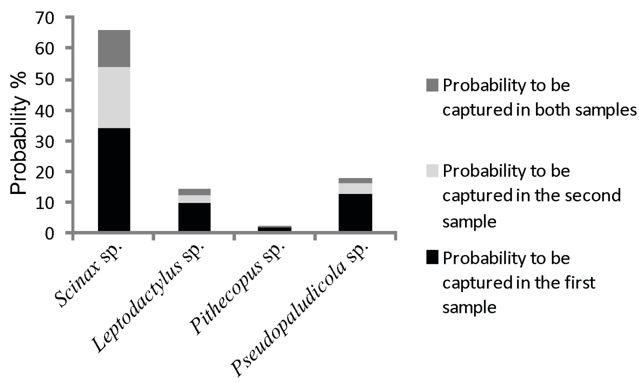

Figure 3. Populations of tadpoles based on descriptors of the Venn diagram according to the temporal sampling.

Table 1. Values for the Kolmogorov-Smirnov sampled by genus.

Sample	*Pithecopus* sp.	*Pseudopaludicola* sp.	*Leptodactylus* sp.	*Scinax* sp.
	4	4	4	4
Maximum deviation	0.2500	0.2500	0.2660	0.2149
Unilateral critical value (0.05)	0.5650	0.5650	0.5650	0.5650
Unilateral critical value (0.01)	0.6890	0.6890	0.6890	0.6890
p(value) unilateral	ns	ns	ns	ns
Bilateral critical value (0.05)	0.6240	0.6240	0.6240	0.6240
Bilateral critical value (0.01)	0.7340	0.7340	0.7340	0.7340
p(value) bilateral	ns	ns	ns	ns

ns = non significant.

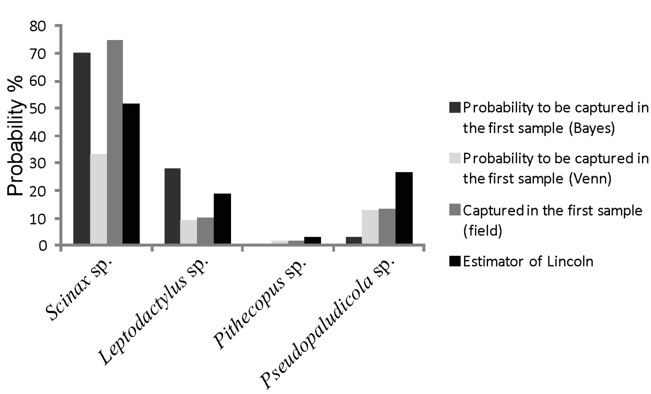

Figure 4. Comparison of the estimators of Lincoln; Bayes theorem and Venn diagram.

populations in water bodies, in this case the population was open making the estimation difficult (Suzuki et al., 2010), unlike studies on tadpoles in lentic environments, where it is possible to control some parameters that directly influence the population size, i.e., emigration and immigration (Abrunhosa et al., 2006), apart from that, the short interval of the study is not influenced by mortality or birth. In northeastern Brazil, where most of the water bodies are temporary (Santos, 2009), the capture-recapture estimation method can be an important tool in the study of anurocenose (Duellman and Trueb, 1994).

We used the Brillouin index to calculate the diversity, because it is a robust index since it has no variance, and no statistical test is necessary to demonstrate significant differences (Magurran 1988, 2011). Furthermore, it is recommended to use this index when the communities are known, and that is why it is used to measure closed diversity, as the parasitic ones (Alves and Luque, 2006; Chaves and Luque, 1999; Luque et al., 2008; Paraguassú and Luque, 2007). If we consider that the community is known in this study because of the limitations in the area of dispersion of populations of *Scinax* sp.; *Leptodactylus* sp.; *Pseudopaludicola* sp.; *Pithecopus* sp., we conclude that the index is ideal for measuring diversity in temporary ponds, which corresponded to 0.136123 in this study. According to Peet (1974) the mathematical assumption must be between $N/S \leq 1.44$ e $X \leq 0.05$ which correspond to the results found in this study.

The Lincoln-Petersen estimator is considered the simplest of population estimates descriptors; the axioms that the model is linked are: (a) closed population – during all research the population remains unchanged; (b) all animals have the same probability of capture in the first sample; (c) the marks do not affect the animal; (d) the second sample is random; (e) during the interval between the samples the animals remain marked (Abuabara and Petrere Junior, 1997; Badii et al., 2012; Krebs, 1989).

Despite the simplicity of the mathematical model of the Lincoln-Petersen estimator, this original equation is the basis for several other estimators with greater complexity, and its mathematical simplicity meets the axiom (Fernandez, 1995). Furthermore, this model when combined becomes robust, and with low bias (Alpizar-Jara and Pollock, 1996).

The Lincoln-Petersen estimator tends to overestimate the population when the sample is small; according to Krebs (1989) that bias can be minimized when the number of trapped animals is greater than seven. In this study, the lowest sample corresponded to 40 individuals, whose recaptured tagged totaled 16 specimens; therefore, it eliminates the bias of overestimation of the population. Begon (1979) believes that the bias can be avoided with the use of a modification of the estimator proposed by Bailey (1951), and ensures more accurate the results. In this case, it was clear that the higher the value m_2, the lower the value of N. When m_2 is small or equal to zero, N is infinite. Therefore, the estimator Lincoln-Petersen shows infinite expectancy and variance. After the modification of the estimator Lincoln-Petersen by Chapman (1951), and Bailey (1951), the average and variance become finite (Abuabara and Petrere Junior, 1997; Zanzini, 2008).

The standard error was 62.19 and 60.95, being the differences lower than the tabulated α (0.94), thus the population calculated between the two estimators (Lincoln-Petersen, and Bailey) do not differed in size. This result reinforces the understanding according to Krebs (1989).

The precision of the Venn descriptors in the model of two samples is coupled to the sample size A n1 and the sample size B n2 (Wittes, 1972). If the relationship between n1 and n2 is too small, the estimation of Ne will show an unacceptable error (Jolly, 1982). In this case, the value of the samples should be raised in the way that the value of Ne approach to the real value of N (Dunn and Andreoli, 1994). In this study, the difference between the first and the second sample corresponded to 10%, and according to Wittes (1972) to satisfy the value of Ne, in relation to the real value of N, the sum of n1 and n2 should correspond to 5% of N, at least. Therefore, in the present study, the diagram of Venn, according to the sampled values higher than the minimum, attend to the recommended axiom.

There is no a capture-recapture process if it is not observed a marked animal during the sample (Silveira Neto et al., 1976). As observed, the estimator of Lincoln-Petersen do not exist when m=0. In contrast, the estimator of Bailey (1951) tends to zero when m tends to zero. Therefore, in the Bayesian focus, the a priori knowledge of the population size, in relation to the proportion of the genus of anuran larvae, as well as the probability $P = (P_{1;} P_{2; \ldots} P_n)$ of larvae capture, being S≥2 the sampling time intervals was the theoretic applied herein to the Bayes theorem. This prior information is a priori distribution of the parameters of Bayesian model, and when no information is available, we can use an a priori uninformative distribution to P and N (Smith, 1991).

Considering the a priori knowledge provided by classical models developed here, as well the Venn diagram, the measured values were the a priori values applied in the Bayes theorem (Smith, 1991; Tierney and Kadane, 1986). Garthwaite et al. (1995) established that the criteria to be adopted for the choice of the prior distribution for Bayesian model can be constituted by non-informative knowledge, since the sample size is known. In this case, we consider the sample size proportions calculated by the classical and Venn methods developed in this study.

For Garthwaite et al. (1995) the corollary to two assumptions should be considered: (i) the probability of capture to a particular animal is the same in each sample, which implies that samples are taken under identical and controlled conditions. For this first corollary the conditions are met in materials and methods through the description of the method in the sample unit; (ii) the catch will be identical and independent, with a known distribution, thus we have to have a good idea of the sampling distribution of the probability of capture. Therefore, through the estimators previously calculated the proportion of the genus *Scinax* sp., *Leptodactylus* sp., *Pithecopus* sp., and *Pseudopaludicola* sp. were maintained, occurring only quantitative variation in each method, that do not represent significant difference according to the Kolmogorov-Smirnov test.

A combination of methods to estimate the population increases strength and reduces the bias of the results (Alpizar-Jara and Pollock, 1996; Pollock, 1991). In the case of an animal presents less chance of being caught, considering all samples, Ne can be calculated for each group and then add the estimates, i.e., when an animal is more likely to be captured in a sample than in another, we may combine the two estimates to determine one (Sekar and Deming, 1949).

The three tested estimators achieved the same level of effectiveness in measuring population size, representing important tools for the study of diversity, quantification and sharing of lentic environments by populations of tadpoles. Together with the marking methodology, which did not change the behavior of tadpoles, the present study helps to fill the need of more studies on larval phase of amphibians in Brazil, especially in its semi-arid northeast.

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