SALAMANDRA **58**(3): 193–202
15 August 2022 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

# Sample size effects, Hill numbers, and trophic niches in anurans

Jorge Mario Herrera-Lopera[1,2], Eduardo Pineda[3], Mirco Solé[4,5] & Carlos A. Cultid-Medina[1,6]

[1)] Instituto de Ecología, A. C. Centro Regional del Bajío. Red de Diversidad Biológica del Occidente Mexicano, Av. Lázaro Cárdenas No. 253, Col. Centro, 61600 Pátzcuaro, Michoacán, México

[2)] Universidad de Caldas, Semillero de Investigación en Biodiversidad y Conservación de paisajes Urbanos (OIKOS), Calle 65 No 26-10, Manizales, Caldas, Colombia

[3)] Instituto de Ecología, A.C. Red de Biología y Conservación de Vertebrados, Carretera antigua a Coatepec 351, El Haya, 91073 Xalapa, Veracruz, Mexico

[4)] Universidade Estadual de Santa Cruz, Laboratório de Herpetologia Tropical. CEP 45662-900 Ilhéus, Bahia, Brazil

[5)] Zoologisches Forschungsmuseum Alexander Koenig, Herpetology Section, Adenauerallee 160, 53113 Bonn, Germany

[6)] Investigador por México (CONACYT), Avenida Insurgentes Sur 1582, 03940 Mexico City, Mexico

Corresponding author: Carlos A. Cultid-Medina, ORCID: 0000-0002-4929-8405, e-mail: carlos.cultid@inecol.mx

Manuscript received: 17 November 2021
Accepted: 6 June 2022 by Stefan Lötters

**Abstract.** Niche breadth and niche overlap measurements have been widely used together to assess resource dynamics in biological communities. However, the estimations of these measurements are susceptible to bias due to sample size. Alternatives such as standardization of niche breadth have been implemented to try and mitigate this bias, but standardization does not solve the underlying problem. In anuran trophic ecology, sampling is usually incomplete, which constrains comparisons. A solution to this problem could lie in implementing an analysis using Hill's series (also called Hill numbers) for comparisons under the same sample coverage; however, this approach has not yet been tested in anuran trophic ecology research. The objectives of this work were to evaluate the bias resulting from sample size for the most frequently used measurements of niche breadth and niche overlap in anuran trophic ecology vis-à-vis overlap measurements derived from Hill's series and to provide a least biased protocol for anuran trophic ecology evaluations. We built data matrices with similar features to those expected for anuran assemblages and quantified the bias of each measurement for different sample sizes. We found that Hill's series measurements were less biased and more informative than traditional ones. We provide an analysis guideline based on Hill's series that facilitates direct comparisons between predator species regarding their consumed and shared prey communities.

Key words. Amphibia, trophic ecology, bias estimation, data simulation, measuring diversity, effective number of prey animals, niche analysis protocol, trophic interactions.

## Introduction

Niche breadth and niche overlap have been used as complementary measurements in descriptions of resource use in biological communities (Krebs 1989, Solé & Rödder 2010). However, both of these have limitations, including the scale at which resources are measured, the spatial distribution of samples, the way that resources are categorized (Colwell & Futuyma 1971, De Cáceres et al. 2011), and the sample size effect (Ricklefs & Lau 1980, Smith & Zaret 1982, Kovács & Török 1997). The latter is especially important because it may change the biological interpretation of the niche breadth and overlap measurements (e.g., underestimated niche breadth or overlaps in species assemblages due to low sample sizes) (Krebs 1989, Kovács & Török 1997). Although the bias resulting from sample size niche breadth measurements was not yet evaluated at the time, Kovács & Török (1997) suggested that for niche breadth comparisons, regardless of the measurement used, a minimum number of samples for each species should be set for their biological interpretation to be meaningful. Based on simulations, Smith & Zaret (1982) then evaluated the sample size effects on seven niche overlap measurements, for which the Morisita and Renkonen measurements were the least and the most biased, respectively, in the niche overlap estimation at minimum sample size (~40 samples). These authors attributed this bias to the weight lent by each measurement to the resource categories within the sample, and to the evenness of these categories in the sample. It is important to note that Smith & Zaret (1982) excluded some of the overlap measurements most frequently used in ecology research, such as Pianka's sym-

metric index, which is the most commonly used measurement in trophic ecology research of amphibian and reptile communities, and for which the estimated bias due to variations in sample size has not been evaluated (Toft 1980, Krebs 1989, Solé & Rödder 2010).

Trophic interactions in anuran communities are useful ecological models for assessing bias in niche breadth and overlap measurements, because compared to other vertebrate groups, anuran diets generally show (i) a comparatively low number of prey animalsconsumed per individual ($\bar{x}$ ~9.85); (ii) a relatively low number of prey categories per species ($\bar{x}$ ~9.75); and (iii) that the minimum number of samples needed for a complete sampling is around 80–115. These sample sizes are often not achieved in short-term samplings because most species' activity periods are restricted to specific seasons and are closely related to climatic variations (Toft 1980, Zug 1993, Kovács & Török 1997, Parmelee 1999, Maneyro et al. 2004, Vitt & Caldwell 2014).

Niche breadth measurements are conceptual applications of alpha diversity measurements (Krebs 1989). The difference between alpha diversity and niche breadth measurements is that the latter are not used to quantify species diversity (as are alpha diversity measurements), but the richness of resource types used by a taxon (Colwell & Futuyma 1971, Krebs, 1989). On the other hand, niche overlap measurements are a beta diversity measurement application (Krebs, 1989). The difference is that overlap measurements are not used to quantify distances in species composition and abundance between two or more communities, but quantify the proportion of resources shared by two or more taxa (Colwell & Futuyma 1971, Krebs 1989).

The most frequently used niche breadth measurements for anuran diet analyses are the Shannon and Levins measurements (Levins, 1968, Colwell & Futuyma 1971, Solé & Rödder 2010). Levins' measurement is equal to the inverse of Simpson's index, which, like Shannon's measurement, is an index used to evaluate community structure. However, applying these indices (based on relative abundances) in trophic ecology research is fraught with the same problem as assessing the diversity of a biological community: they lack biologically interpretable units. For example, Shannon index units depend on the logarithm used. Thus, the magnitude of the Shannon index does not explicitly reveal how the community is structured according to the distribution of relative abundances across species or resource items (e.g., prey) (see MacArthur 1965). The absence of biological units (e.g., species or operational taxonomic units, OTUs) also limits unbiased comparisons between units of analysis (see below). Hill (1973) addressed the cautioning remarks by MacArthur (1965) and proposed that the diversity or structure of a community (i.e., how relative abundance is distributed among species) be assessed from a mathematical series that has since become known as Hill's numbers or the Hill's series. The Hill's numbers formula is presented in detail in the Materials and methods section of this paper, but it may be helpful to highlight that: i) Hill's numbers allow to estimate community diversity in biologically interpretable units (i.e., the effective number of species); ii) Hill's main numbers or diversity orders are N = 0, which estimates species richness, N = 1 is the effective number of equally common species, equivalent to the exponential of Shannon's index, and N = 2 estimates the effective number of abundant or dominant species, equivalent to the reciprocal of Simpson's index; and iii) that they offer a unified mathematical framework for the comparison of diversity among communities (Hill 1973, Jost 2006).

The most used overlap measurement in anuran trophic ecology research is Pianka's (1973) symmetric measurement, which is mathematically similar to MacArthur's and Levins's overlap measurement, except that the latter is asymmetrical (Pianka 1974, Krebs 1989, Solé & Rödder 2010). Other measurements less widely employed for trophic niche analysis are Renkonen's percentage overlap or Schoener's measurement (Krebs 1989), and Morisita's similarity measurement, which has been suggested to be the least biased by sample size (Smith & Zaret 1982). Additionally, since the niche breadth measurements most frequently used in anuran dietary analysis are derivatives of the orders 1 and 2 of Hill's series, the species turnover measurements for the diversity orders 1 and 2 (Jost 2007) could be used.

A recurrent aim in trophic ecology studies of anuran communities is the comparison between species (e.g., Toft 1980, Parmelee 1999, Solé & Rödder 2010). However, niche breadth measurements cannot be compared directly, because their value ranges depend on the number of each species' resources (Krebs 1989). To address this problem of comparability, many authors have proposed the standardization of niche breadth measurements, adjusting their value range between 0 and 1 (Krebs 1989, Maneyro et al. 2004, Lima, Rödder & Solé 2010, Solé & Rödder 2010, Forti et al. 2011, Marín-Martínez et al. 2019). However, since the most commonly used measurements of niche breadth (i.e., Shannon and Levins) can be obtained from Hill's series, comparisons can (and should) be made considering the concept of sample coverage (Chao & Jost 2012).

Sample coverage can be understood as "the actual relative abundance of the community represented by the pool of species in a sample" and can be used as a criterion for the comparison of samples at the same level of completeness through an interpolation/extrapolation protocol (Chao & Jost 2012, Chao et al. 2014). Furthermore, when comparing measurements at the same level of sample coverage, the sample size bias is minimized by default (Chao & Jost 2012, Engel et al. 2021), allowing direct comparison of niche breadth measurements between species independent of species abundance. However, this concept has not yet been used in anuran trophic ecology studies (or in any other biological group, to our knowledge). The sample coverage approach should be applied to comparisons of beta diversity, too (overlap in the case of anuran diets). However, how exactly this concept should be applied in this area is not fully resolved, and its application is still under development (Engel et al. 2021).

Our general objective was to evaluate the bias due to changes in sample size in the measurements most frequently used in dietary assessments of anurans and compare it with values obtained from the Hill's series framework. Considering that sample size effects have not yet been evaluated for niche breadth measurements but been limited to a few niche overlap measurements, and given that achieving a minimally informative sample number for the study of anuran trophic ecology is a central challenge, we address the following questions: (i) how does the ecological interpretation differ in the analysis of prey communities of frog species using traditional niche breadth measurements from the Hill's series approach?; (ii) how do the ecological interpretations differ in the analysis of the trophic niche overlap of a frog assemblage using a traditional overlap and Hill's series approach?; (iii) how does the bias in the estimation of trophic niche breadth and overlap for simulated anuran species change with variations in sample size?; and (iv) which are the least biased breadth and overlap measurements for dietary descriptions of anurans? Therefore, we here provide a suitable dietary analysis protocol for anurans considering their trophic characteristics and sensitivity to sample size.

## Materials and methods

### Comparison of approaches

To compare traditional and Hill's series measurements to evaluate niche breadth and overlap, we used diet data obtained for an assemblage of four frog species inhabiting avocado orchards in central-western Mexico (*Eleutherodactylus angustidigitorum*, *E. nitidus*, *Dryophytes arenicolor* and *Lithobates forreri*). Details of these diets can be found in the Table S1. We considered as traditional niche breadth and niche overlap measurements those compiled by Solé & Rödder (2010). For niche breadth we used two measurements, the standardized Shannon's measurement, which is mathematically equal to Pielou's measurement of evenness:

$$J' = \frac{-\sum p_i \log p_i}{\log n} \quad (1)$$

where 'J'': standardized Shannon's measurement; $p_i$: proportional abundance of each prey category; and n: number of prey categories; and the standardized Levins's niche breadth measurement, which is equivalent to the standardized Simpson's index:

$$B_A = \frac{\left(\frac{1}{\sum p_i^2}\right)-1}{n-1} \quad (2)$$

where $B_A$: standardized Levins' measurement, $p_i$: relative abundance of each prey or category; and n: number of prey categories.

As traditional niche overlap measurement, we used Pianka's symmetric index which can be calculated as:

$$O_{jk} = \frac{\sum p_{ij} p_{ik}}{\sqrt{\sum_i^n p_{ij}^2 \sum_i^n p_{ik}^2}} \quad (3)$$

where $O_{jk}$ is the niche overlap and $p_{ij}$ and $p_{ik}$ represent the $i^{th}$ resource proportion used by 'j' and 'k' species.

Niche breadth measurements were compared directly. For their calculation we used the R environment package 'abdiv' (Bittinger 2020). The Pianka's symmetric index was calculated using the R environment package 'spaa' (Zhang 2016).

For description of prey communities based on Hill's series, we applied the Jost (2006) mathematical notation:

$$^qD = \left(\sum_{i=1}^s p_i^q\right)^{1/(1-q)} = \left(^q\lambda\right)^{1/(1-q)} \quad (4)$$

where $p_i$ is the $i^{th}$ prey proportional abundance, S is the prey richness, and q is the diversity order (for orders different from 1). When q = 1, diversity should be calculated as:

$$^1D = \exp\left(\sum_{i=1}^s p_i \log p_i\right) \quad (5)$$

and it is equivalent to the exponential Shannon's measurement.

To compare prey diversity among anuran species, we used 0, 1 and 2 diversity orders (i.e., $^0D$, $^1D$ and $^2D$), and the comparison was carried out with equal sample coverage:

$$\hat{C}_n = 1 - \frac{f_1}{n}\left[\frac{(n-1)f_1}{(n-1)f_1+2f_2}\right] \quad (6)$$

where $\hat{C}n$ is the sample coverage, $f_1$ is "singletons" (prey with abundance equal to 1), $f_2$ is "doubletons" (prey with abundance equal to 2), and n is the total number of prey-registered individuals. Sample coverage allows the estimation of the proportion of the community represented by the prey in the sample; using the same sample coverage level allows a direct comparison of diversity order values (Chao & Jost 2012). To evaluate differences in the estimations, we used comparisons on the 95% confidence interval overlaps (Cumming, Fidler & Vaux 2007), estimated via bootstrap (10,000 repetitions) (Legendre & Legendre 2012). The $^qD \pm 95\%$ CI per frog species was estimated using the 'iNEXT' package of R (Hsieh et al. 2016).

For overlap measurements, we used $1 - {}^qD_\beta$ in orders 0, 1 and 2 (i.e., $^0D_\beta$, $^1D_\beta$ and $^2D_\beta$; modified from Jost (2007):

$$^1D_\beta = 1 - \left[\left(^q\lambda_\gamma / {}^q\lambda_\alpha\right)^{1/(1-q)}\right] = {}^q\lambda_\alpha{}^{1/(1-q)} \quad (7)$$

where $^q\lambda_\alpha$ is the order q alpha diversity measurement (local diversity, in this case single-species prey diversity), $^q\lambda_\gamma$ is the order q gamma diversity measurement (landscape diversity, in this case all-species prey diversity), and q is the diversity order (for orders different from 1). When q =

1, beta diversity should be calculated as the q limit to 1 (see Jost 2007 for a wider explanation). For the ${}^{q}D_{\beta}$ calculation, we used the 'vegetarian' package of the R environment (Charney & Record 2012).

To evaluate differences in estimations of overlaps between species pairs, we conducted visual comparisons based on 95% confidence intervals (Cumming, Fidler & Vaux 2007), estimated via bootstrap (10,000 repetitions) (Legendre & Legendre 2012).

### Selecting niche breadth and overlap measurements

The niche breadth measurements evaluated in this work were both of the orders ${}^{1}D$ (equivalent to Shannon's exponential measurement) and ${}^{2}D$ (equivalent to Levins' niche breadth measurement and the inverse of Simpson's index) proposed by Hill (1973). For measuring overlaps, we used Pianka's symmetric measurement, overlap percentage (Renkonen's measurement), Morisita's (similarity) overlap measurement (Krebs 1989), and 1 – turnover measurements for diversity orders 1 and 2 of the Hill's series framework (Jost 2007). For all measurements, species × prey category sum vectors were considered, which were constructed from anuran individuals × prey category input matrices.

### Input matrices

For niche breadth, we considered hypothetical species with two known trophic niche breadth values for each measurement, an intermediate (${}^{1}D$: 6.42; ${}^{2}D$: 5.04) and a maximum value, equal to prey item richness (${}^{1}D$: 10; ${}^{2}D$: 10). We considered species with 10 prey categories and a mean prey number of 10 individuals. For this purpose, we used the 'rpois' function of the R environment (R Core Team 2021) with lambda = 10 and n = 10 to generate a vector of 10 random numbers (prey categories). From the generated vector we constructed abundance matrices of 200 anuran individuals (maximum sample size).

For overlap measurements, two hypothetical species with 15 prey categories in total (10 for each species) were considered in two scenarios: (i) five shared prey categories (overlap ~50%); (ii) all prey categories shared (overlap ~100%). We considered a mean prey number of 10 individuals and used the 'rpois' function (R Core Team 2021) to generate two vectors with a 15 random numbers vector (prey categories) for each frog species. From the generated vectors we constructed two frog species abundance matrices of 200 individuals each (maximum sample size per species considered here).

### Simulation procedure

The following procedure was used both for the amplitude and niche overlap simulation protocols. Sum vectors were generated by randomly choosing anuran individuals from the input matrices, with sample sizes of 5, 10, 20, 40, 80, 160 and 200 individuals. We calculated 5,000 times the measurement for each sample size using random sum vectors. Then, we estimated: (i) the mean of the estimation for each sample size; (ii) the standard deviation for each estimation for each sample size; (iii) the 95% confidence intervals for each sample size; and (iv) the bias of the estimation for each sample size, calculated as:

$$\text{bias} = 1 - \frac{\text{observed value}}{\text{expected value}} \qquad (8)$$

where the 'observed value' was the value of the mean for each sample size, and the 'expected value' was the known value of the sum vector initially generated. This bias calculation takes values between 0 and 1 and can be transformed to a percentage. All analyses and the functions used for our simulations were built in the R environment version 4.1.2 (R Core Team 2021), using the 'vegetarian' (Charney & Record 2012) and 'spaa' (Zhang 2016) packages.

## Results

The results when comparing the trophic niche breadth of the frog assemblage using the traditional and the Hill's series approaches were different (Fig. 1). The traditional approach detected no differences between species' niche breadths (Figs 1A–B). The Hill's series-derived approach detected differences between species' trophic niches at ${}^{0}D$, ${}^{1}D$ and ${}^{2}D$ (Figs 1C–E). Regarding trophic niche overlap measurements, there were no significant differences between species pairs using the Pianka's symmetric index (Fig. 2A). Likewise, there was no significant difference between species pairs using ${}^{1}D_{\beta}$ and ${}^{2}D_{\beta}$, while ${}^{0}D_{\beta}$ detected statistically significant differences between *Dryophytes arenicolor* and *Lithobates forreri* and the other species pairs (Figs 2B–D).

All niche breadth and overlap measurements evaluated in this work exhibited biases with respect to sample size (Fig 3. 3, 4, Table S2). As for niche breadth, with the ${}^{1}D$ (i.e. Shannon's exponential measurement), the maximum bias for the expected value of 6.42 was 80.70% (Fig. 5A) and for the expected value of 10 the maximum bias was 77.07% (Fig. 5B). With ${}^{2}D$ (Levins' niche breadth measurement), for the expected value of 5.04 the bias was 82.53% (Fig. 5C), and for the expected value of 10 the maximum bias was 78.52% (Fig. 5D).

Likewise, the sample size at which the estimates were stabilized (i.e., where the bias of the expected value is less than 10%) was 80 samples for expected value 10 and 160 samples for expected value 6.41 of the ${}^{1}D$ measurement, and 160 samples for both expected values 5.04 and 10 of the ${}^{2}D$ measurement ( 5).

For the niche overlap measurements, the maximum bias occurred at low sample size values (5–10 samples, Figs 4, 6), and ranged between 58 and 82%. In decreasing bias or-

der, the less biased measurements were: $^2D_\beta$ (58.57% for 0.5 expected overlap, and 60.55% for 1 expected overlap; Figs 6I–J), $^1D_\beta$ (64.96% for 1 expected overlap, and 65.82% for 0.5 expected overlap; Figs 6G–H), Pianka's symmetric measurement (71.97% for 1 expected overlap, and 76.21% for 0.5 expected overlap; Figs 6E–F), Morisita's measurement (72.94% for 0.5 expected overlap, and 82.27% for 1 expected overlap; Figs 6C–D) and Renkonen's overlap percentage (76.47% for 0.5 expected overlap, and 81.25% for 1 expected overlap; Figs 6A–B).

The overlap measurements that stabilized faster (first reaching bias values of less than 10%) with increasing sample size were: $^1D_\beta$ (40 samples for 1 expected overlap, and 80 samples for 0.5 expected overlap), $^2D_\beta$ and Pianka's symmetric measurement (80 samples, for both 0.5 and 1 expected overlap), Morisita's measurement (80 samples for 1 expected overlap, and 160 samples for 0.5 expected overlap) and Renkonen's overlap percentage (160 samples for both 0.5 and 1 expected overlap; Fig. 6).

## Discussion

Hill's series for trophic ecology analysis of anuran assemblages allow a more comprehensive and realistic biological view of the observed patterns than do those obtained

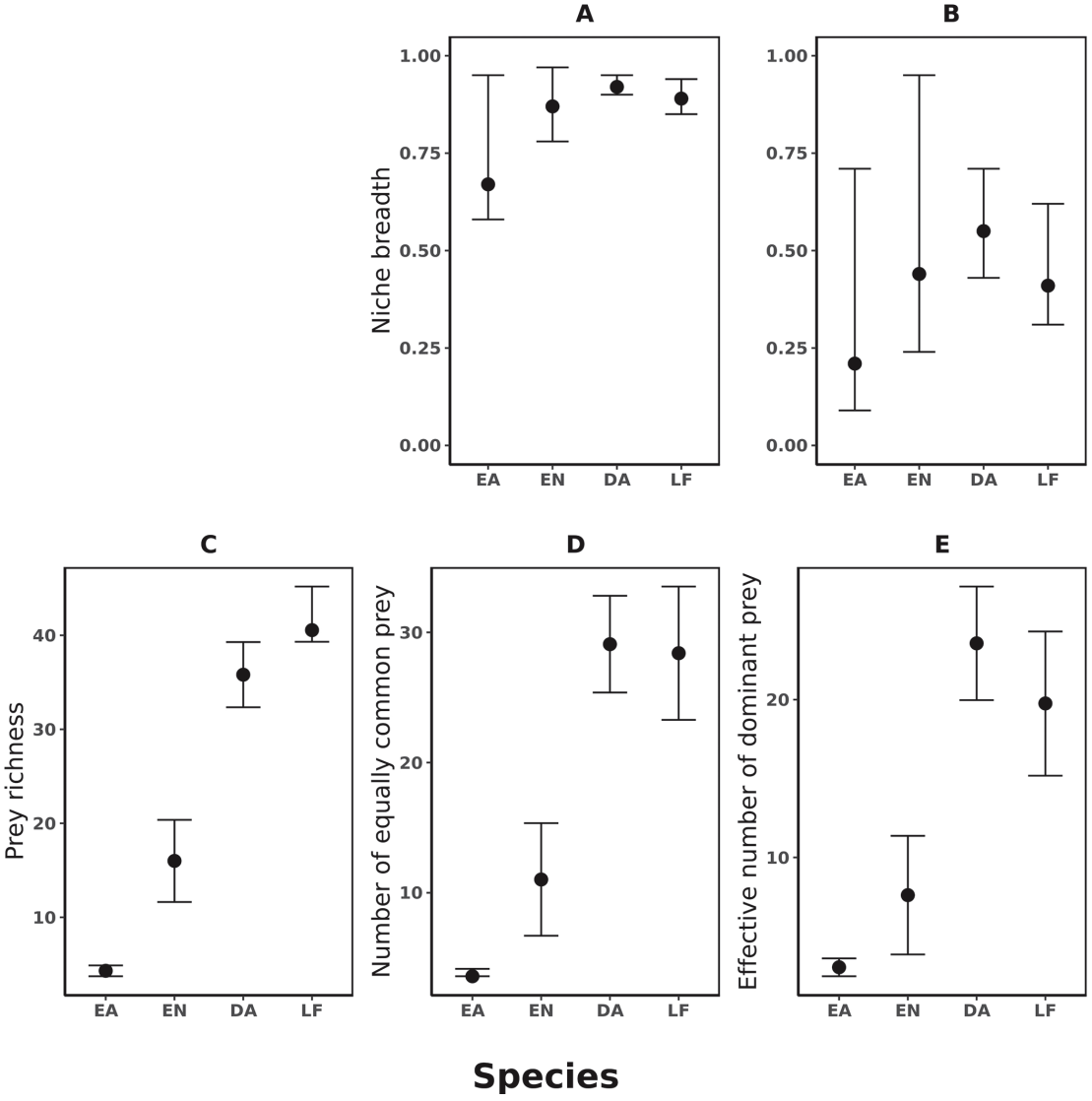


Figure 1. Evaluation of the prey community consumed by an anuran assemblage in avocado orchards in central-western México (mean ± 95 % CI). (A) Shannon's standardized measurement of niche breadth; (B) Levins' standardized measurement of niche breadth; (C) $^0D$ of Hill's series, equivalent to prey richness; (D) $^1D$ from Hill's series, equivalent to the effective number of equally common prey items; (E) $^2D$ of Hill's series, equivalent to the effective number of dominant prey. EA: *Eleutherodactylus angustidigitorum*, EN: *Eleutherodactylus nitidus*, DA: *Dryophytes arenicolor*, LF: *Lithobates forreri*.

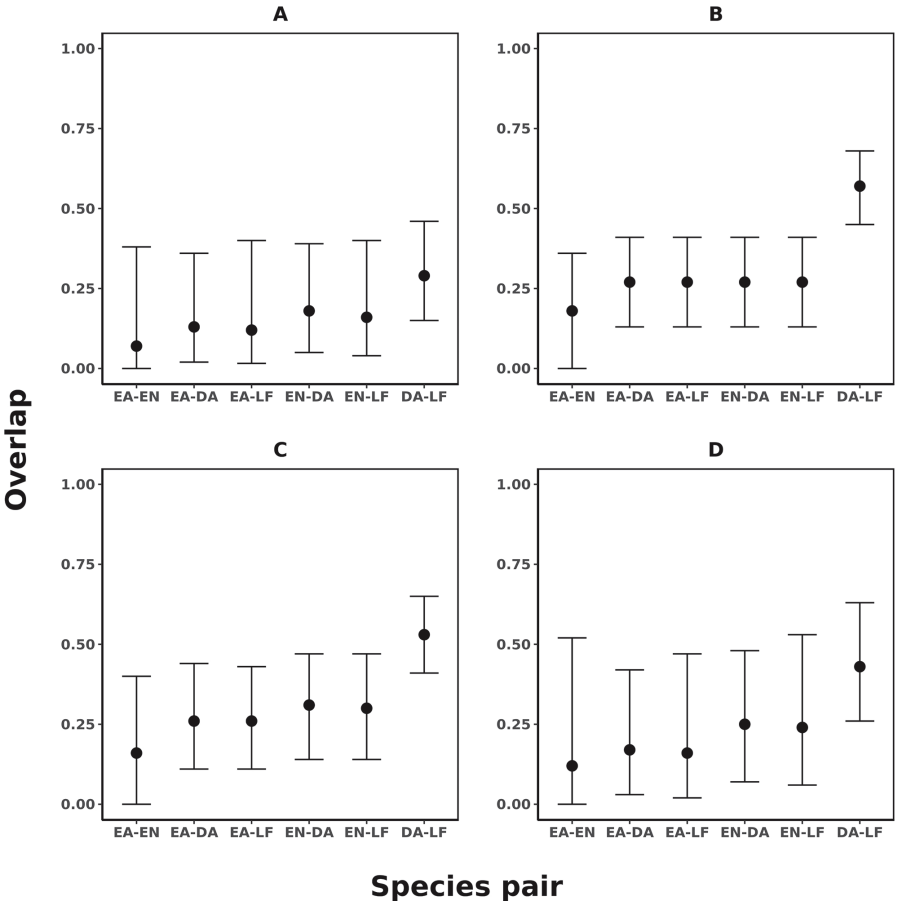


Figure 2. Overlap evaluations in a frog assemblage in avocado orchards in central-western México through distinct methods (mean ± 95 % CI). (A) Pianka's symmetric measurement; (B) ${}^{0}D_{\beta}$, equivalent to overlap by prey richness; (C) ${}^{1}D_{\beta}$, equivalent to overlap by equally common prey; (D) ${}^{2}D_{\beta}$, equivalent to overlap by dominant prey. EA: *Eleutherodactylus angustidigitorum*, EN: *Eleutherodactylus nitidus*, DA: *Dryophytes arenicolor*, LF: *Lithobates forreri.*

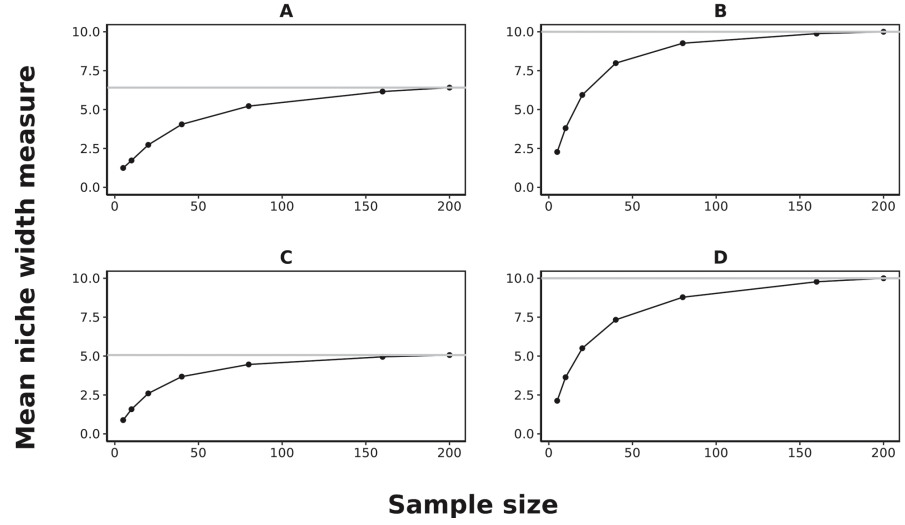


Figure 3. Variations in the trophic niche breadth estimation with respect to sample size. Left: Expected values 6.41 above and 5.06 below. Right: Expected value for both cases 10. (A–B) ${}^{1}D$ (i.e., Shannon's exponential); (C–D) ${}^{2}D$ (i.e., Levins' niche breadth measurement, cf. Simpson's inverse index). Grey line: Expected value for each case.

through the use of traditional measurements of niche breadth and overlap. Our results suggest that all niche breadth and niche overlap measurements evaluated were biased at small sample sizes (5–10 samples) and that bias decreased when sample size increased. For niche breadth measurements, the percentage of bias difference was less than 3%. For overlap, the bias percentage differed between measurements, with ${}^{1}D_{\beta}$ and ${}^{2}D_{\beta}$ being the least biased ones and their difference to the next least biased measurement (Pianka's symmetric measurement) was approximately 7%, and about 10% to the most biased overlap measured (Renkonen measurement). Regarding estimation stabilization (a sample size where the bias is less than 10%), the earliest niche breadth measurement to stabilize was ${}^{1}D$, while for the overlap one it was ${}^{1}D_{\beta}$.

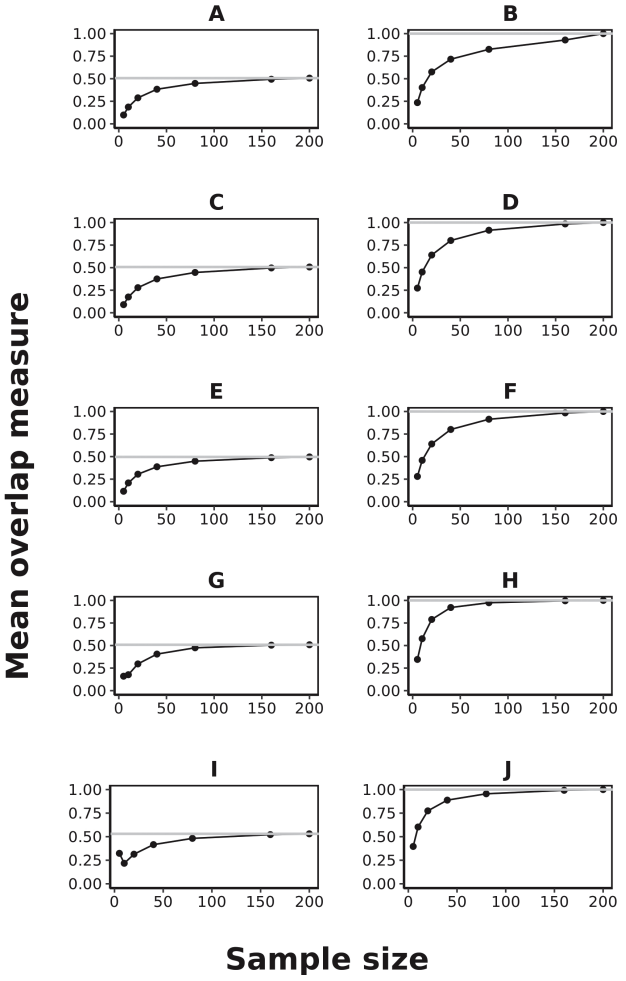


Figure 4. Mean overlap estimations across different sample sizes for different measurements. Left: Estimate when half of the composition is shared (overlap ~ 50%). Right: Estimation when all of the composition is shared (overlap~100%). (A–B) Percentage overlap (Renkonen, 1938); (C–D) Morisita (1959); (E–F) Pianka's symmetrical measurement (Pianka, 1973); (G–H) ${}^{1}D_{\beta}$ (Jost 2007); (I–J) ${}^{2}D_{\beta}$ (Jost 2007). Grey line: Expected value for each case.

The interpretations of trophic analyses of anurans from the traditional approach were different from those using the Hill's series approach. According to the observations with the traditional approach, there were no significant differences between the trophic niche amplitudes in any of the species in the anuran community, while in the approach using the Hill's series and the comparison based on sampling coverage, differences in the prey community consumed by each species were observed in three ways: (i) in the prey richness consumed by the species of the assemblage, forming three different groups; (ii) three differentiated groups of species were formed according to their equally common prey; and (iii) two differentiated groups of species were formed according to the effective number of their dominant prey.

Krebs (1989) and Solé & Rödder (2010) suggested employing a standardized trophic niche breadth (mathematically adapted so that their value domain stays between 0 and 1) to compare species. However, standardization limits the actual variation observed and may cause misinterpretation when comparing the niche breadth between two or more species. Trophic niche breadth measurements are alpha diversity measurements (i.e., orders 1 and 2 of Hill's series), so that Chao et al.'s (2014) suggestion for alpha diversity comparisons is applicable to them: the communities (species in this case) should be compared at the same percentage of sample coverage to avoid biological misinterpretations due to sample size effects. The use of sample coverage in anuran diet analysis permits the comparison of anuran diet diversity (i.e., niche breadth) even if their sample sizes are different, if they are at the same level of sample coverage (Kovács & Török 1997, Chao et al. 2014).

The bias between overlap measurements was variable. The ${}^{1}D_{\beta}$ and ${}^{2}D_{\beta}$ estimators were less biased and required fewer samples to reach less than 10% bias. On the other hand, the Morisita's measurement and the Renkonen's overlap percentage were the most biased estimators with respect to sample size. These results contrast with Smith & Zaret (1982), who found that the Morisita's measurement was the less biased measurement in the estimation of overlap with respect to sample size. This may be because Smith & Zaret (1982) used a minimum sample size of 50 samples, while our study used a minimum sample size of 5 samples, and we found that Morisita's measurement bias decays by between 40–60% when sample size increases from 5 to 50 samples. Smith & Zaret (1982) and Krebs (1989) suggested that the most biased overlap measurement with respect to sample size is Renkonen's overlap percentage. This result was consistent with our findings in that Renkonen's overlap percentages had the highest-percentage biases of all overlap measurements with respect to sample size and was the measurement that required the largest number of samples to stabilize (i.e., to reach a bias value of less than 10%).

Pianka's (1974) statement that the use of distinct overlap measurements is "somewhat arbitrary since similar qualitative results are obtained with a wide variety of indices" can be considered correct, because all measurements

underestimate overlaps at small sample sizes (5–10 samples), but this underestimation (bias) was not the same for all overlap measurements. Our data suggest that both Pianka's and Morisita's overlap measurements should not be used for sample sizes smaller than 80 individuals and Renkonen's overlap measurement should not be used with sample sizes smaller than 160 individuals. For sample sizes smaller than 80, the most recommendable measurements (due to their lower biases) are ${}^{1}D_{\beta}$ and ${}^{2}D_{\beta}$ (see above).

We found differences in the ecological interpretations derived from traditional analysis approaches to the description of prey communities in anuran assemblages and in their overlap analyses. In general, the Hill's series approach facilitated visualization and comparison in a manner that traditional measurements did not (i.e., richness, equally common prey and dominant prey), so that interpretation and the description of anuran trophic interactions become more thorough. Additionally, due to the implementation of the comparison by sampling coverage, a sample-size bias in comparisons is avoided (Chao & Jost 2012), allowing prey communities to be compared directly and without the need to use standardizations that distort observed patterns.

The ${}^{1}D$ and ${}^{2}D$ measurement biases were similar. However, since these are equivalent to orders 1 and 2 of the Hill's series, their use should not be mutually exclusive, but rather complementary, because each one reflects a different portion of diversity (trophic niche) (Hill 1973, Jost 2006). Regarding the interpretation of each measurement (i.e., order of diversity) in an anuran dietary context, ${}^{1}D$ would reflect the equally abundant dietary components, without bias for rare or abundant prey, and ${}^{2}D$ reflects the most abundant (i.e., dominant) dietary components in the prey community that are consumed by the anuran species (Jost 2018, Cultid-Medina & Escobar 2019).

Due to sample size bias results found in both niche breadth and niche overlap measurements, and because Hill's series approach provides a more adequate trophic niche quantification and biological interpretation, we propose the following dietary analysis guidelines for anurans:

1. Prey diversity consumed by anuran species (trophic niche) should be described using the diversity measurements ${}^{0}D$: Consumed prey richness, ${}^{1}D$: Number of equally common prey (equivalent to Shannon exponential measurement), and ${}^{2}D$: Effective number of dominant prey items (equivalent to Levins niche breadth measurement).

2. Comparison of any of the diversity orders (i.e., ${}^{0}D$, ${}^{1}D$ and ${}^{2}D$) should be carried out when species are at the same level of sample coverage, or by interpolating or extrapolating their values to ensure that the comparison is at the same level (see Chao et al. 2014 for an explanation of the interpolation/extrapolation protocol).

3. Anuran dietary similarity (overlap) should be compared using as measurements the inverse Jost (2007) turnover (i.e., ${}^{1-q}D_{\beta}$) of orders 0, 1 and 2 as follows: ${}^{0}D_{\beta}$: overlap by prey richness, ${}^{1}D_{\beta}$: overlap by equally common prey, and ${}^{2}D_{\beta}$: overlap by dominant prey.

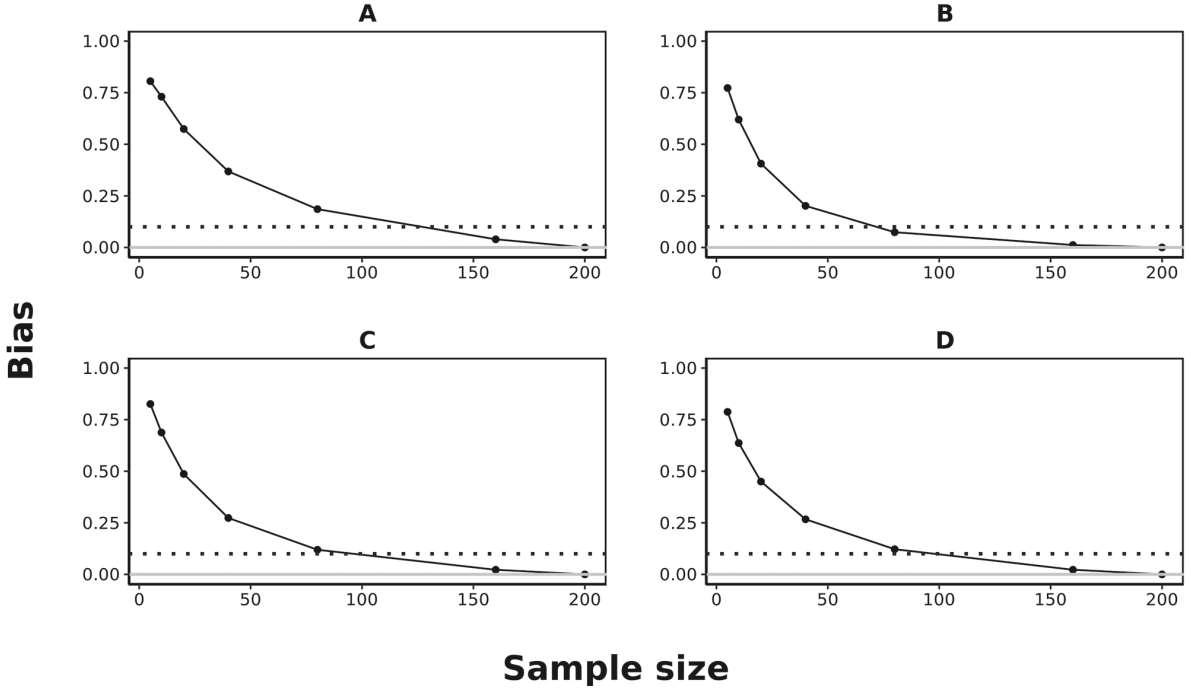


Figure 5. Bias in the estimation of trophic niche breadth as a result of sample size. Bias is estimated as abs (1 – estimated value/expected value). (A–B) ${}^{1}D$ (i.e., Shannon's exponential); (C–D) ${}^{2}D$ (i.e., Levins' trophic niche breadth measurement, i.e,. inverse Simpson index). Grey line: Expected value for each case, dotted line: 10% bias.

4. Although overlap measurements in anuran diets should be compared when the sampling effort (sample coverage) is the same for all species (as beta diversity comparisons), the manner in which sample coverage should be applied to beta comparisons is not fully resolved (Engel et al. 2021), and its resolution is beyond the scope of this paper.

5. Niche breadth and overlap measurements should be accompanied by complementary and more descriptive ones such as the index of relative importance (IRI) (Pinkas et al. 1971; Hart et al. 2002), to arrive at more accurate and complete interpretations of the trophic dynamics of anuran communities than those obtained by measuring trophic niche breadth and overlap only.

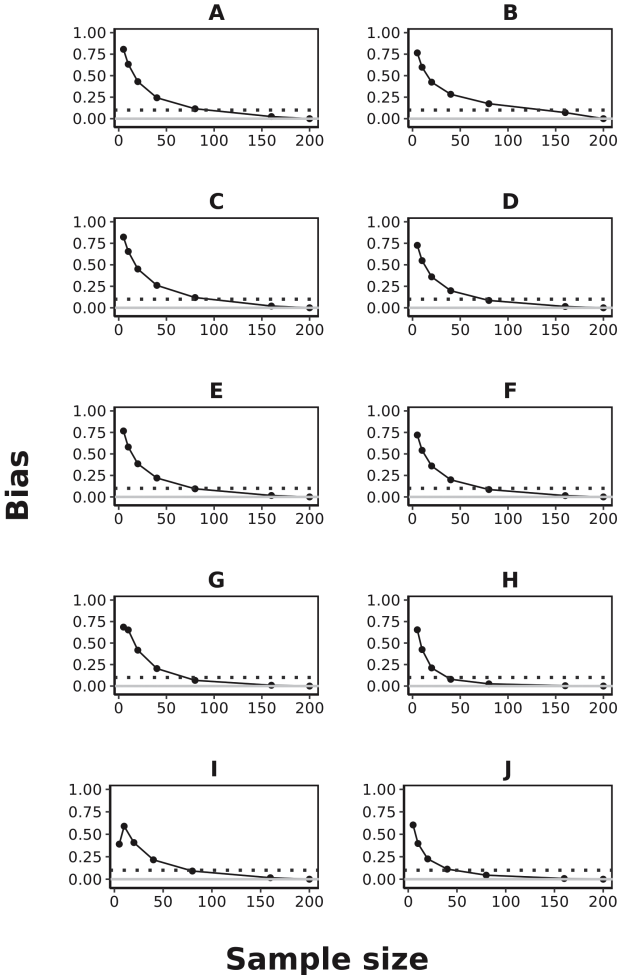


Figure 6. Bias in overlap estimation for different measurements at different sample sizes. The bias is estimated as abs (1 – estimated value/expected value). Left: Estimation when half of the composition is shared (overlap ~50%), right: Estimation when all of the composition is shared (overlap ~100%). (A–B) Percentage overlap (Renkonen 1938); (C–D) Morisita (1959); (E–F) Pianka's symmetrical measurement (Pianka 1973); (G–H) $^{1}D_{\beta}$ (Jost 2007); (I–J) $^{2}D_{\beta}$ (Jost 2007). Dotted line: 10% bias.

**Acknowledgements**

This work was part of JMHL's Master in Science thesis, funded by CONACyT with a scholarship for postgraduate studies at the Instituto de Ecología, A.C. (INECOL, scholarship number 753814). This study is part of the development of the Cátedra CONACyT project (No. 673: 2017-2027), in which Dr. Cultid-Medina is a co-researcher (https://bpnlab.wordpress.com). We thank Armando Aguirre, Ireri Suazo, Karoline Ceron, Sonia Gallina, and Roger Guevara for their valuable comments and suggestions on early versions of this paper.

**References**

Bittinger, K. (2020): Abdiv: alpha and beta diversity measures. R package version 0.2.0. – Online document, available at: https://CRAN.R-project.org/package=abdiv, accessed 31 May 2021.

Chao, A., N. J. Gotelli, T. C. Hsieh, E. L. Sander, K. H. Ma, R. K. Colwell & A. M. Ellison (2014): Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. – Ecological Monographs, **84**: 45–67.

Chao, A. & L. Jost (2012): Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. – Ecology, **93**: 2533–2547.

Charney, N. & S. Record (2012): Vegetarian: Jost diversity measures for community data. R package version 1.2. – Online document, available at: https://CRAN.R-project.org/package=vegetarian, accessed 31 May 2021.

Colwell, R. K. & D. J. Futuyma (1971): On the measurement of niche breadth and overlap. – Ecology, **52**: 567–576.

Cultid-Medina, C. A. & F. Escobar (2019): Pautas para la estimación y comparación estadística de la diversidad biológica (qD). – pp. 175–202 in: Moreno, C. E. (ed.): La biodiversidad en un mundo cambiante: Fundamentos teóricos y metodológicos para su estudio. – Universidad Autónoma del Estado de Hidalgo, Pachuca de Soto.

Cumming, G., F. Fidler & D. L. Vaux (2007): Error bars in experimental biology. – Journal of Cell Biology, **177**: 7–11.

De Cáceres, M., D. Sol, O. Lapiedra & P. Legendre (2011): A framework for estimating niche metrics using the resemblance between qualitative resources. – Oikos, **120**: 1341–1350.

Engel, T., S. A. Blowes, D. J. McGlinn, F. May, N. J. Gotelli, B. J. McGill & J. M. Chase (2021): Using coverage-based rarefaction to infer non-random species distributions. – Ecosphere, **12**: e03745.

Forti, L. R., A. S. O. Tissiani, T. Mott & C. Strüssmann (2011): Diet of *Ameerega braccata* (Steindachner, 1864) (Anura: Dendrobatidae) from Chapada dos Guimarães and Cuiabá, Mato Grosso State, Brazil. – Brazilian Journal of Biology, **71**: 189–196.

Hart, R. K., M. C. Calver & C. R. Dickman (2002): The index of relative importance: an alternative approach to reducing bias in descriptive studies of animal diets. – Wildlife Research, **29**: 415–421.

Hill, M. O. (1973): Diversity and evenness: a unifying notation and its consequences. – Ecology, **54**: 427–432.

Hsieh T. C., K. H. Ma & A. Chao (2016): iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). – Methods in Ecology and Evolution, **7**: 1451–1456.

Jost, L. (2006): Entropy and diversity. – Oikos, **113**: 363–375.

Jost, L. (2007): Partitioning diversity into independent alpha and beta components. – Ecology, **88**: 2427–2439.

Jost, L. (2018): What do we mean by diversity? The path towards quantification. – Mètode Revista de difusió de la investigació. **9**: 39–45.

Kovács, T. & J. Török (1997): Determination of minimum sample size to estimate diet diversity in anuran species. – Herpetological Journal, **7**: 43–48.

Krebs, C. (1989): Ecological methodology, 2nd edition. – Harper & Row, New York.

Legendre, P. & L. Legendre (2012): Numerical ecology, 3rd English edition. – Elsevier, Amsterdam.

Levins, R. (1968): Evolution in changing environments, some theoretical explorations. – Princeton University Press, Princeton.

Lima, J. E. P., D. Rödder & M. Solé (2010): Diet of two sympatric *Phyllomedusa* (Anura: Hylidae) species from a cacao plantation in southern Bahia, Brazil. – North-Western Journal of Zoology, **6**: 13–24.

MacArthur, R. H. (1965): Patterns of species diversity. – Biological Reviews, **40**: 510–533.

Maneyro, R., D. E. Naya, I. da Rosa, A. Canavero & A. Camargo (2004): Diet of the South American frog *Leptodactylus ocellatus* (Anura, Leptodactylidae) in Uruguay. – Iheringia, Série Zoologia, **94**: 57–61.

Marín-Martínez, M., M. S. Cárdenas-Ortega, J. M. Herrera-Lopera & J. A. Rojas-Morales (2019): Diet of the yellow striped poison frog, *Dendrobates truncatus* (Cope, 1861) (Anura: Dendrobatidae) from the Middle Magdalena river valley, Colombia. – Herpetology Notes, **12**: 1185–1191.

Moreno, C. E. (2019): La biodiversidad en un mundo cambiante; fundamentos teóricos y metodológicos para su estudio. – Universidad Autónoma del Estado de Hidalgo, Pachuca de Soto.

Parmelee, J. R. (1999): Trophic ecology of a tropical anuran assemblage. – Natural History Museum The University of Kansas, **11**: 1–59.

Pianka, E. R. (1973): The structure of lizard communities. – Annual Review of Ecology and Systematics, **4**: 53–74.

Pianka, E. R. (1974): Niche overlap and diffuse competition. – Proceedings of the National Academy of Sciences, **71**: 2141–2145.

Pinkas, L., M. S. Oliphant & I. L. K. Iverson (1971): Food habits of albacore, bluefin tuna, and bonito in California waters. – Fisheries Bulletin, **152**: 11–105.

R Core Team (2021): R: a language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna.

Ricklefs, R. E. & M. Lau (1980): Bias and dispersion of overlap indices: results of some Monte Carlo simulations. – Ecology, **61**: 1019–1024.

Smith, E. P. & T. M. Zaret (1982): Bias in estimating niche overlap. – Ecology, **63**: 1248–1253.

Solé, M. & D. Rödder (2010): Dietary assessments of adult amphibians. – pp. 167–184 in: Kenneth Dodd Jr. C. (ed.): Amphibian ecology and conservation: a handbook of techniques. – Oxford University Press, Oxford.

Toft, C. A. (1980): Feeding ecology of thirteen syntopic species of anurans in a seasonal tropical environment. – Oecologia, **45**: 131–141.

Vitt, L. J. & J. P. Caldwell (2014): Herpetology: an introductory biology of amphibians and reptiles, 4th edition. – Elsevier, Amsterdam and Boston.

Zhang, J. (2016): Spaa: SPecies Association Analysis. R package version 0.2.2. – Online document, available at: https://CRAN.R-project.org/package=spaa, accessed 31 May 2021.

Zug, G. R. (1993): Herpetology: an introductory biology of amphibians and reptiles. – Academic Press, San Diego.

## Data availability statement

The R language code for the different analyses, as well as the matrices employed, are available at: https://github.com/carlosculTid84/Hill_NicheTrophic/blob/main/RCode_Salamandra_Final.zip, or by contacting the authors.

## Supplementary data

The following data are available online:

Supplementary document S1. Species assemblage used for the comparison of approaches.

Supplementary document S2. Tables of means, standard deviations, confidence intervals, and percentage biases for each measurement assessed in this work.