

Efficient vs. structured biodiversity inventories: reptiles in a Mexican dry scrubland as a case study

S. Trujillo–Caballero, J. A. González–Oreja

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Abstract

Efficient vs. structured biodiversity inventories: reptiles in a Mexican dry scrubland as a case study. Many sampling methods allow the study of species richness and diversity in biological communities, but it is not known whether a single method can determine both the number and diversity of species in an unbiased and efficient way. Here we assess whether the least biased and most efficient method to determine reptile species richness in a Mexican dry scrubland is also the best method to estimate species diversity. The local assemblage was composed of 10 species, with the Mexican mud turtle (*Kinosterton integrum*) and the Jalapa spiny lizard (*Sceloporus jalapae*) being the dominant ones. Microhabitat surveys (MHS) were the most accurate and the most efficient method to estimate species richness, but they over-estimated species diversity (+67.1%) as much as the other sampling methods, i.e., transect surveys and pitfall-trap stations, under-estimated it (–59%). Our study shows that the best sampling method to determine the number of species in local assemblages may not be the best method to study species diversity. Although combining different sampling methods can increase the project costs in terms of time, effort and money, the use of structured inventories is recommended for the analysis of species diversity.

Key words: Biodiversity knowledge, Number of species, Number of equiprobable species, Sampling methods, Sampling effort, Pitman efficiency

Resumen

Comparación entre inventarios de biodiversidad eficientes y estructurados: los reptiles de un matorral xerófilo de México como ejemplo. Se han propuesto muchos métodos para estudiar la riqueza y la diversidad de especies en comunidades biológicas, pero se desconoce si existe alguno que pueda determinar tanto el número como la diversidad de especies sin sesgo y de forma eficiente. En este estudio evaluamos si el método menos sesgado y más eficiente para determinar la riqueza de reptiles en un matorral xerófilo de México es también el mejor para estimar la diversidad de especies. La comunidad local estaba compuesta por 10 especies, de las que la tortuga de pecho quebrado y pata rugosa (*Kinosterton integrum*) y la lagartija escamosa jalapeña (*Sceloporus jalapae*) eran las dominantes. Los muestreos en microhábitats (MHS) fueron el método más exacto y eficiente para estimar la riqueza de especies, pero sobrestimaron (+67,1%) la diversidad de especies tanto como la subestimaron (–59%) los demás métodos (i.e., los itinerarios y las estaciones de trampas de caída). Nuestro estudio muestra que el mejor método de muestreo para determinar el número de especies en las comunidades locales tal vez no sea el mejor para estudiar la diversidad de especies. Aunque combinar diferentes métodos de muestreo puede aumentar el tiempo, el esfuerzo y los costos económicos relacionados con los proyectos, recomendamos el uso de inventarios estructurados para analizar la diversidad de especies.

Palabras clave: Conocimiento de la biodiversidad, Número de especies, Número de especies equiprobables, Métodos de muestreo, Esfuerzo de muestreo, Eficiencia de Pitman

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Saúl Trujillo–Caballero, José Antonio González–Oreja, Fac. de Biología. BUAP, Benemérita Univ. Autónoma de Puebla, Edificio 112–A, Ciudad Universitaria, 72570 Puebla, México.

Corresponding author: José Antonio González–Oreja. E–mail: jgonzorj@hotmail.com

Introduction

Historically, the concept of biodiversity has been nearly equivalent to the number of species in a local assemblage (Sarkar, 2002; Tucker, 2005; Leitner and Turner, 2013). Knowing species richness is a legitimate objective in ecology and conservation biology (Colwell and Coddington, 1994; Gotelli and Colwell, 2001; Leitner and Turner, 2013), but equality between biodiversity and species richness is inaccurate (Swingland, 2013). In addition to the total number of species, the measurement of biodiversity should include information about the relative importance (equitability) of the species in the studied assemblage (Gotelli and Colwell, 2011). Fortunately, the toolbox of the modern biodiversity student includes many measures that consider species equitability (Magurran, 2005; Jost and González–Oreja, 2012; Gotelli and Chao, 2013).

Many factors can influence the measurement of species richness and diversity (Hill et al., 2005; Magurran, 2005), such as sampling effort (Colwell and Coddington, 1994; Gotelli and Colwell, 2001; Gotelli and Chao, 2013) and the effects that the sampling method has on the fraction of biodiversity sampled (Hill et al., 2005; Sutherland, 2006; Eekhout, 2010; McDiarmid et al., 2012). To increase the set of truly sampled species and to obtain a more accurate estimation of the total richness, biodiversity students can combine (i.e., add up) data obtained with different sampling methods. This practice is known as a structured inventory (Gotelli and Ellison, 2013) and it has frequently been applied in biodiversity studies (for instance, see King and Porter, 2005; Coddington et al., 2009; Gotelli et al., 2011, or Castro et al., 2017, for arthropods; Jenkins et al., 2003; Hutchens and DePerno, 2009; Sung et al., 2011; Foster, 2012, or Carpio et al., 2015, for amphibians and reptiles; or Pech Canche et al., 2011, for bats).

Nevertheless, because of systematic biases in favor of or against certain activity periods, animal behaviors or body sizes (Hutchens and DePerno, 2009), the different sampling methods applied in a structured inventory can differ in their probability to capture different species (Yoccoz et al., 2001). As a result, differences may arise in the relative abundance of the registered species (Longino et al., 2002; Coddington et al., 2009) and lead to contrasting estimates of species diversity obtained with varied sampling methods.

Reptiles are ectothermic animals, and the study of reptile biodiversity is not only strongly influenced by their biology, physiology and behavior (Willmer et al., 2005; McDiarmid et al., 2012; Vitt and Caldwell, 2014) but also by the environmental variables which constrain them (i.e., temperature or humidity; Latham et al., 2005). All these factors can involve different capture probabilities for the same species with different sampling methods (Hutchens and DePerno, 2009; Rodda, 2012). Since the election of a good sampling method can be a critical factor in a biodiversity study (Cardoso, 2009), we asked the following question: can the best method to es-

timate species richness simultaneously be the best method to estimate species diversity? To the best of our knowledge, this is the first time this question has been posed in the biodiversity literature. In this paper, using our data on the reptile assemblage in a Mexican dry scrubland, we (i) compared the performance (in terms of bias and efficiency) of three sampling methods frequently used in reptile biodiversity studies (Eekhout, 2010; McDiarmid et al., 2012): microhabitat surveys, transect surveys, and pitfall–trap stations; and (ii) analyzed whether the most accurate and most efficient (i.e., the best) method to estimate the number of species in our local reptile assemblage was, at the same time, the best method to study a measure of diversity that considers species equitability.

Material and methods

Study area

Field work was conducted at Tecali de Herrera (Puebla, Mexico: 18° 48' 24" – 18° 57' 54" N, 97° 57' 54" – 98° 05' 42" W), at an altitude of ca. 2,000. The climate is temperate (mesothermic) and subhumid (average values for 1951–2010: annual temperature, ca. 17 °C; total annual precipitation, ca. 600 mm), and rain is mostly recorded during summer (SMN, 2015). Since insolation is high and humidity is low, evapotranspiration can reach elevated values (mean total annual evaporation for the period 1951–2010: 1,890 mm). Because of anthropic changes in land use, most of the original vegetation has disappeared (SEGOB, 2006) and the landscape is currently covered by an open, dry scrubland over diverse soil types and geological substrates (Rzedowski, 1988; Saldaña Munive, 2011). Several cacti (e.g., *Echinocactus* sp., *Mammillaria* sp.), tree-like cacti (*Stenocereus* sp.), and other succulent plants (like *Agave salmiana*, *A. stricta*, and *Yucca periculosa*) were dominant in this landscape. Finally, because of the extreme climatic conditions, crop fields were scarce, and several kilometers away from where we completed our field work.

Field work

From June 2005 to April 2006, we completed monthly reptile inventories using the following methods (Latham et al., 2005; Blomberg and Shine, 2006): (1) microhabitat surveys, (2) transect surveys, and (3) pitfall–trap stations.

Microhabitat surveys (MHS) are the simplest method to capture small reptiles (Blomberg and Shine, 2006). We actively searched for reptiles in sites (i.e., microhabitats) that are usually preferred as refuges by the considered species (McDiarmid et al., 2012), such as under rocks, fallen logs, metal sheets and the bark of tree trunks, or between cracks. All specimens were captured by hand. The total number of MHS was 23, distributed throughout the whole study period (no less than once per month; table 1s in supplementary material).

Transect surveys (TS) consisted of two itineraries (1 km each) across the study area. These itineraries intersected several small, temporal or permanent creeks. Transect surveys were sampled at low pace, first between 8 and 9 a.m., and then between 4 and 5 p.m., 23 times in total (table 1s in supplementary material). We recorded all the species observed along each TS, as well as other data on their activity.

Pitfall-trap stations (PFS) were modified from the arrangement recommended by Crosswhite et al. (1999). Each PFS consisted of four pitfall-traps (45 cm deep, with the bottom perforated to reduce the risk of drowning by rain water accumulation), plus six double-ended funnel-traps covered with a cylindrical screen ($\varnothing = 35$ cm), all connected by three drift fences (10 m long, buried in the ground to a depth of 10 cm, and with additional 50 cm above the ground). PFS, with the pitfall-traps partially covered with leaves to reduce the risk of death due to the high insolation, were open for 24 h. To limit the stress to trapped reptiles, we checked traps twice a day (morning and midday). Throughout the study, we used appropriate tools to collect and handle reptiles (e.g. rubber bands, snake hooks, nets), and identified all captured individuals by using the keys of Casas and McCoy (1979) and Flores Villela et al. (1995).

We completed only 13 PFS, all from June to October 2005, because our PFS suddenly 'disappeared' from the study area. Therefore, we split the analyses into two time periods: (1) data from June to October 2005 (the initial period) allowed us to compare, with low sampling efforts, MHS, TS and PFS; (2) data from June 2005 to April 2006 (all our field work) allowed us to compare only MHS and TS, but with higher sampling efforts.

Measuring reptile biodiversity

With the data obtained from all the three sampling methods from the first period, or only from MHS and TS throughout all our field work (table 1s in supplementary material), we computed the following descriptors of reptile biodiversity (Magurran, 2005).

Richness (S): number of species, computed with individual-based rarefaction or extrapolation techniques, after standardization of all the methods compared to a common number of individuals captured (Gotelli and Colwell, 2001). Modern individual-based richness estimation techniques through extrapolation allowed to compute the expected number of species that could be obtained with a total abundance above the number really observed (Colwell et al., 2012; Gotelli and Chao, 2013). However, these techniques offer credible results if the total number of individuals is below $3\times$ (or, even better, $2\times$) the actual total abundance (Colwell et al., 2012). Therefore, to assess the quality of the inventories in the first period, we compared the observed richness per method with the one computed by extrapolation to 164 individuals; and, in all our field work, up to 224 individuals (i.e., $2\times$ the corresponding total abundance: $n = 82$ individuals for the first period, and $n = 112$ throughout all our field work). To compare

richness values obtained with different numbers of individuals, we used the more traditional rarefaction technique (Gotelli and Colwell, 2001). We present values computed by rarefaction from n individuals as S_n , and those by extrapolation as S_n^* ; for example, S_{35} is the number of species expected in a sample of 35 individuals, computed by rarefaction, whereas S_{246}^* is the number of species expected in a sample of 246 individuals, computed by extrapolation.

True species diversity (1D): number of equiprobable species (Jost, 2006; Jost and González-Oreja, 2012; Moreno et al., 2011), computed with the total species abundances from each sampling method (i.e., MHS vs. TS vs. PFS), or with the sum of the abundances from several, pooled methods (vg., MHS + TS): ${}^qD = [\sum p_i^q]^{1/(1-q)}$, where p_i is the proportion of individuals of species i , and q is the order of the diversity measure. Since we used $q = 1$, the previous formula was equivalent to $\exp[-\sum p_i \times \ln(p_i)]$ (Jost, 2006; see Cruz Elizalde and Ramírez Bautista, 2012 for the same approach in a reptile biodiversity study). We also standardized sampling effort to a common number of individuals, which allowed us to compare true diversity measures by using modern resampling techniques (Colwell, 2013). We present expected diversity measures computed this way from a sample of n individuals as 1D_n ; for instance, ${}^1D_{35}$ is the mean number of equiprobable species expected in a sample of 35 individuals, computed by resampling methods. We performed all richness and diversity calculations with *EstimateS* vers. 9.1.0 (Colwell, 2013). To obtain smooth curves for the richness and diversity estimators, we always used $n = 100$ repetitions, with no replacement between individuals.

Assessing performance (bias and efficiency): we followed Walther and Moore (2005) and Zar (2010) to assess the bias of both richness and true species diversity measures obtained with a given sampling effort j (E_j) as the difference between that measure and a reference value, accepted as real (A). For the richness estimates, we used the following expression (fig. 1A): PAR $_j$ (percent of actual richness) = $100 * SME_j + 100$, where SME $_j$ (scaled mean error) = ME_j/A , and ME $_j$ (mean error) = $E_j - A$. For the diversity estimates, instead of PAR, the corresponding term was PAD $_j$ (percent of actual diversity), and all the rest were equivalent. Finally, we considered the asymptote from the smooth richness accumulation curve obtained by pooling all the sampling methods as the best available estimation of true richness (A); in a parallel way, we considered the diversity accumulated with all the sampling methods as the best available estimation of true diversity (Walther and Moore, 2005; Ellison et al., 2007).

We computed the Pitman efficiency index (Das-Gupta, 2005; Zar, 2010) and considered that a sampling method, $M1$, was more efficient than a second method, $M2$, if $M1$ could achieve the same response value (y) as $M2$ with a lower sampling effort (i.e., if $n1 < n2$; fig. 1B). We present the efficiency of $M1$ relative to $M2$ as the ratio between the sample sizes needed to achieve y with both methods (Zacks, 2005): $n2/n1$. If this ratio > 1 , then $M1$ was more

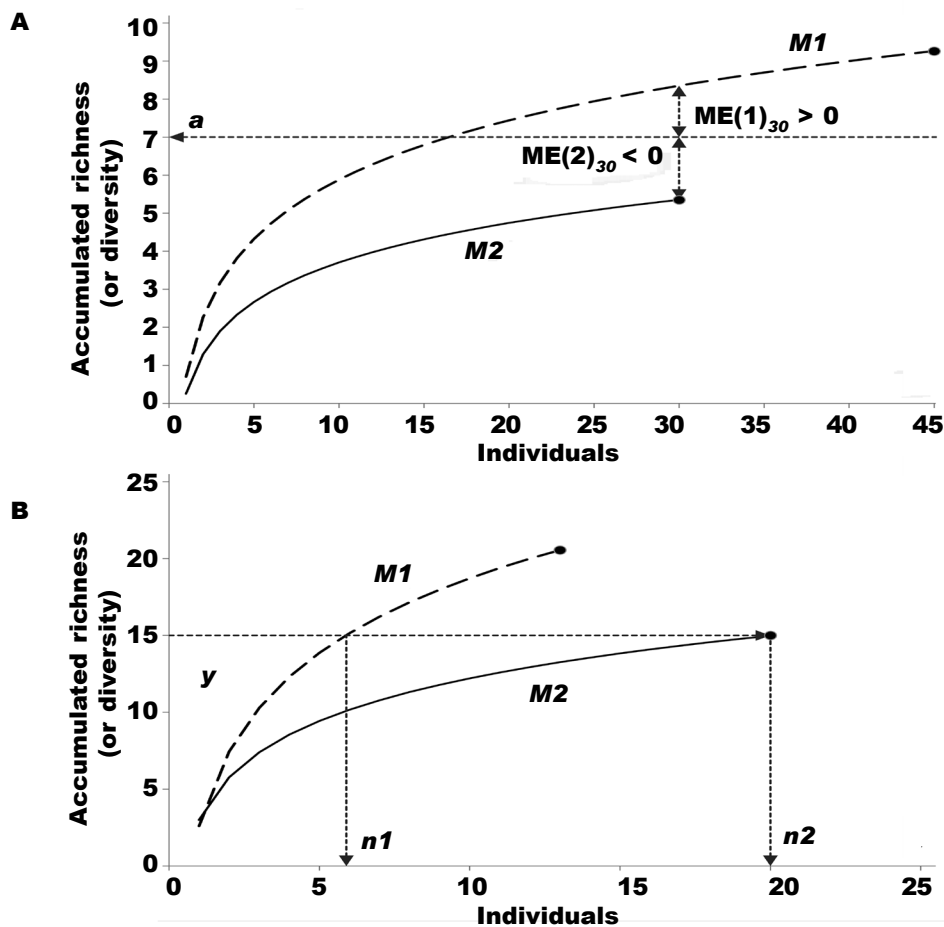


Fig. 1. A, an example of how to measure bias in species richness (or diversity) estimates from two sampling methods, *M1* and *M2*. $ME(1)_{30}$ and $ME(2)_{30}$ are the mean errors in the richness (or diversity) estimates by *M1* and *M2* with a common sampling effort; in this example, 30 individuals. *a* is the reference value, accepted as real, for richness (or diversity); in this example, $a = 7$. B, an example of how to measure the relative efficiency of two sampling methods, *M1* and *M2*. The common response to attain by the two methods, *y*, is the minimum richness (or diversity) accumulated with one of the two methods compared; in this example, $y = 15$. The corresponding sampling efforts to attain the *y* value with the two methods are $n1$ and $n2$.

Fig. 1. A, un ejemplo de cómo cuantificar el sesgo en las estimaciones de la riqueza (o diversidad) de especies calculadas con dos métodos de muestreo: *M1* y *M2*. $ME(1)_{30}$ y $ME(2)_{30}$ son los errores medios de las estimaciones de la riqueza (o diversidad) *M1* y *M2* con un esfuerzo de muestreo común, que en este ejemplo es de 30 individuos. *a* es el valor de referencia, aceptado como real, de la riqueza (o diversidad); en este ejemplo, $a = 7$. B, un ejemplo de cómo cuantificar la eficiencia relativa de dos métodos de muestreo: *M1* y *M2*. La respuesta común de ambos métodos, *y*, es la riqueza (o diversidad) mínima acumulada con uno de los dos métodos comparados; en este ejemplo, $y = 15$. Los esfuerzos de muestreo correspondientes para obtener el valor de *y* con los dos métodos son $n1$ y $n2$.

efficient than *M2*. We assessed the relative efficiency of our sampling methods to describe both species richness and true species diversity; the value of *y* used in each evaluation was the minimum of the total accumulated richness, or total accumulated diversity, obtained with any of the sampling methods being compared (fig. 1B).

Results

Short description of the reptile assemblage

During the first study period (from June to October 2005) we did not find any animals (i.e., we obtained only negative results) in two of 15 MHS, in three of

Table 1. Frequency and abundance of the reptile fauna in the study area, by time periods (initial period, from June 2005 to October 2005; and total period, from June 2005 to April 2006) and sampling methods: MHS, microhabitat surveys; TS, transect surveys; PFS, pitfall-trap stations. Frequency (f) is the number of sampling units where each species was recorded, and abundance (N) is the number of individuals actually recorded: – absences.

Tabla 1. Frecuencia y abundancia de la herpetofauna en la zona de estudio, por períodos (período inicial, de junio de 2005 a octubre de 2005; y período total, de junio de 2005 a abril de 2006) y métodos de muestreo: MHS, muestreos en microhábitats; TS, itinerarios; PFS, estaciones de trampas de caída. La frecuencia (f) es el número de unidades de muestreo en las que se registra cada especie y la abundancia (N) es el número de individuos registrado: – ausencias.

	Time period									
	Initial						Total			
	MHS		TS		PFS		MHS		TS	
	f	N	f	N	f	N	f	N	f	N
<i>Anolis quercorum</i>	1	2	–	–	–	–	2	4	–	–
<i>Aspidoscelis sacki</i>	2	4	5	7	2	7	3	5	8	10
<i>Kinosternon integrum</i>	5	11	–	–	–	–	5	11	–	–
<i>Masticophis mentovarius</i>	1	1	–	–	–	–	1	1	–	–
<i>Phrynosoma braconnieri</i>	2	2	–	–	–	–	2	2	–	–
<i>Salvadora intermedia</i>	3	3	–	–	–	–	3	3	1	1
<i>Sceloporus horridus</i>	1	1	1	1	1	1	2	2	3	3
<i>Sceloporus jalapae</i>	3	5	10	27	3	3	7	10	18	51
<i>Tantilla bocourti</i>	1	2	–	–	–	–	2	3	–	–
<i>Thamnophis cyrtopsis</i>	4	5	–	–	–	–	5	6	–	–

15 TS, and in eight of 13 PFS (supplementary material). The mean number of captured species was 2.1 (range: 1–4) per MHS with positive results; 1.6 (range: 1–3) per positive TS, and 1.2 (range: 1–2) per positive PFS. During this period, we captured 36 individuals with MHS, 35 with TS, and 11 with PFS. The two most frequently captured species in the MHS (table 1) were the Mexican mud turtle (*Kinosternon integrum*) and the Black-necked gartersnake (*Thamnophis cyrtopsis*); they were also the two most abundantly captured species, together with the Jalapa spiny lizard (*Sceloporus jalapae*). The most frequently captured and the most abundant species in the TS was *S. jalapae* followed by Sack's giant whiptail lizard (*Aspidoscelis sacki*). In the PFS, *S. jalapae* was the most frequently captured species and *A. sacki* was the most abundant.

Throughout our field work we did not capture any individuals in six out of 23 MHS (supplementary material); in the positive MHS, the mean number of captured species was 1.9 (range: 1–4). With all the MHS, we captured 47 specimens of the same species captured in the first period with this method (table 1); *S. jalapae*, *K. integrum* and *T. cyrtopsis* were the most frequent, whereas *K. integrum* and *S. jalapae* were the most abundant. In five out of 23 TS we did

not find any individuals (supplementary material); the mean number of species in the positive TS was 1.6 (range: 1–4). With all the TS, we found 65 specimens of the same species captured in the first period with this method, plus the Oaxacan patchnose snake (*Salvadora intermedia*) (table 1); *S. jalapae* and *A. sacki* were the most frequent and abundant species.

Richness

During the first study period, the accumulated richness curve obtained with the sum of abundance data pooled from the three sampling methods was located above the corresponding curve for PFS and below that for MHS; the curve with the smallest slope was that for TS (fig. 2A). The only sampling method that captured all the species recorded in this study was MHS ($S_{36} = 10$). After theoretically doubling the total pooled sampling size, expected richness remained constant ($S_{164}^* = 10.2$; 95% confidence interval, based on the unconditional estimator of standard deviation: 9.1–11.2; fig. 2A).

For all our field work, richness results were like those we presented above. Although using TS we captured a total of 65 individuals (almost 2× the corresponding total abundance from the first period),

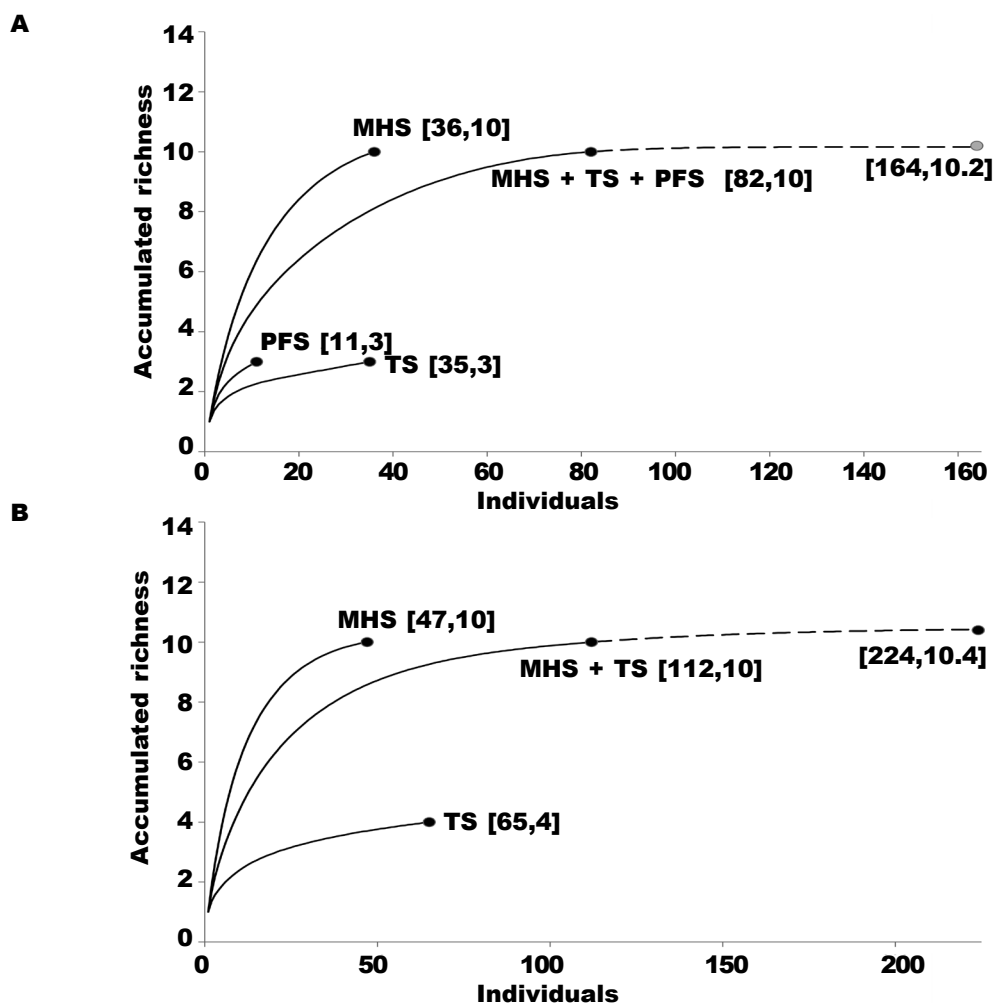


Fig. 2. Smooth, accumulated species richness curves (y -axis: number of species) vs. increasing sampling effort (x -axis: number of accumulated individuals) for: A, initial period (when the three methods were operating); B, all our field work (only for MHS and TS). In each panel, the dotted line shows the extrapolation up to $2\times$ the corresponding total abundance. The total abundance and richness values for each period \times method are shown in brackets: MHS, microhabitat surveys; TS, transect surveys; PFS, pitfall-trap stations.

Fig. 2. Curvas continuas de la riqueza de especies acumulada (eje de las y : número de especies) en relación con un esfuerzo de muestreo creciente (eje de las x : número de individuos acumulados) para: A, el período inicial (cuando se aplicaban los tres métodos); B, todo nuestro trabajo de campo (solo para los MHS y los TS). En cada gráfico, la línea discontinua muestra la extrapolación hasta el doble de la abundancia total correspondiente. Los valores totales de abundancia y riqueza de cada período \times método se muestran entre corchetes: MHS, muestreos en microhábitats; TS, itinerarios; PFS, estaciones de trampas de caída.

the accumulated total richness with this method $S_{65} = 4$ (i.e., just one above the three from the first period), and the accumulated richness curve seemed to finally reach the asymptote (fig. 2B). The accumulated richness with all the MHS did not increase from the value obtained in the first period ($S_{47} = 10$). The total richness accumulated with the sum of these two sampling methods did not increase either ($S_{112} = 10$). After extrapolating this pooled curve up

to $2\times$ the total number of individuals, the increase in expected richness was negligible ($S_{224}^* = 10.4$; 95% confidence interval: 8.4–12.5; fig. 2B).

True species diversity

After pooling data from the three sampling methods, the accumulated species diversity curve (${}^1D_{35} = 5.1$ and ${}^1D_{82} = 5.5$; fig. 3A) was located between

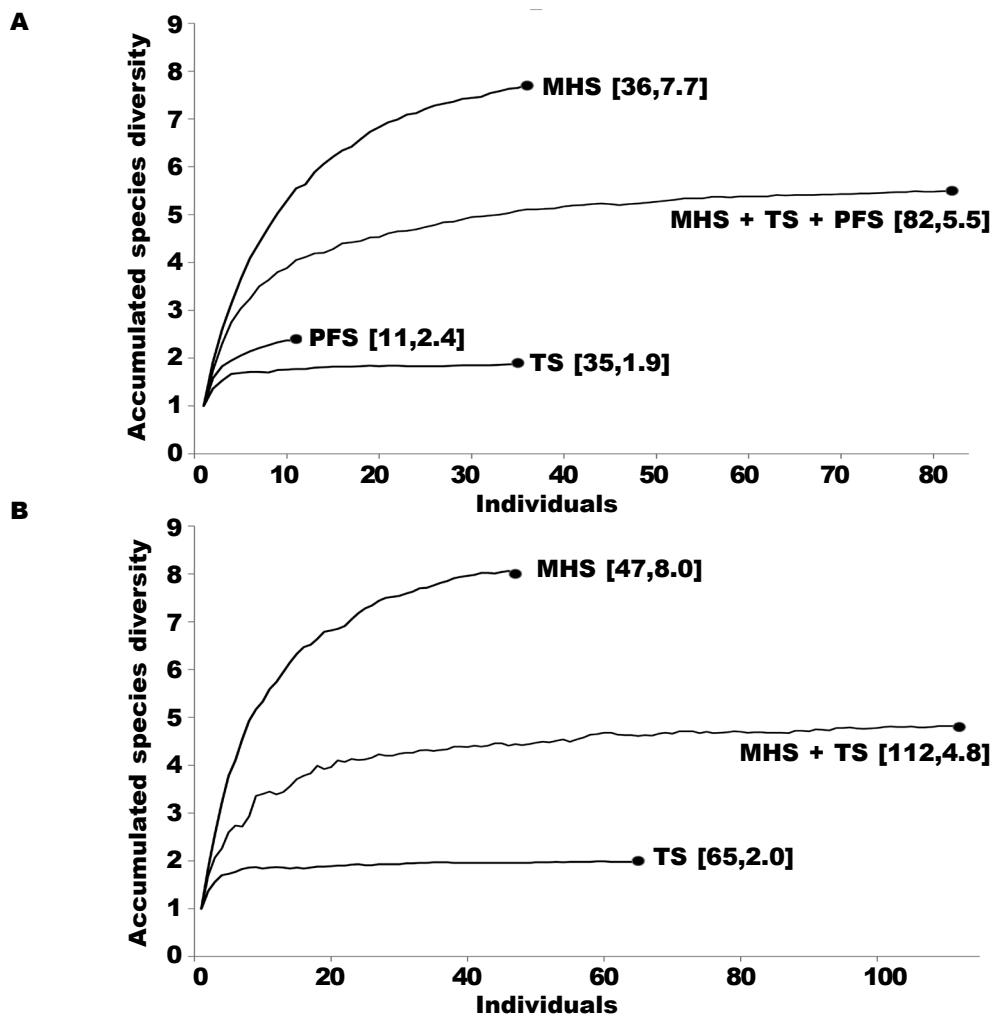


Fig. 3. Smooth, accumulated species diversity curves (y-axis: number of equiprobable species) vs. increasing sampling effort (x-axis: number of accumulated individuals) for: A, initial period (when the three methods were operating); B, all our field work (only for MHS and TS). The total abundance and richness values for each period \times method are shown in brackets: MHS, microhabitat surveys; TS, transect surveys; PFS, pitfall-trap stations.

Fig. 3. Curvas continuas de la diversidad de especies acumulada (eje de las y: número de especies equiprobables) en relación con un esfuerzo de muestreo creciente (eje de las x: número de individuos acumulados) para: A, el período inicial (cuando se aplicaban los tres métodos); B, todo nuestro trabajo de campo (solo para los MHS y los TS). Los valores totales de abundancia y riqueza de cada período \times método se muestran entre corchetes: MHS, muestreos en microhábitats; TS, itinerarios; PFS, estaciones de trampas de caída.

those for MHS (${}^1D_{36} = 7.7$) and PFS (${}^1D_{11} = 2.4$); the first curve to reach the asymptote was that from TS (${}^1D_{35} = 1.9$; fig. 3A). For all our field work, the MHS diversity curve seemed to approach the asymptote (${}^1D_{47} = 8.0$; fig. 3B), and the TS curve did not change from the first period (${}^1D_{65} = 2.0$), even though the total number of individuals did increase (see above). The total species diversity computed with the sum of abundances from all our field work with these two methods (${}^1D_{82} = 4.7$; ${}^1D_{112} = 4.8$)

was slightly inferior to that previously obtained with the individuals captured only during the first study period (i.e., when the three sampling methods were functioning: ${}^1D_{82} = 5.5$; figs. 3A, 3B).

Bias and efficiency

Regarding species richness, MHS was the least biased method: with high sampling efforts, bias was negligible (e.g., for the first study period:

Table 2. Bias (A) and relative efficiency (B) of microhabitat surveys (MHS), transect surveys (TS), and pitfall–trap stations (PFS). Bias was computed as the percentage of actual richness, or diversity, obtained with n individuals. Efficiency was computed as the ratio between the numbers of individuals needed to achieve the given value (richness or diversity) with the two methods compared. As for the initial period 'Sum' was MHS + TS + PFS, but it was MHS + TS for the total: – results not defined.

Tabla 2. Sesgo (A) y eficiencia relativa (B) de los muestreos en microhábitats (MHS), los itinerarios (TS) y las estaciones de trampas de caída (PFS). El sesgo se calculó como porcentaje de la riqueza real, o la diversidad, obtenida con n individuos. La eficiencia se calculó como la proporción entre el número de individuos necesarios para lograr un valor dado (riqueza o diversidad) con los dos métodos comparados. Con respecto al periodo inicial "Sum" fue MHS + TS + PFS, pero para el total fue MHS + TS: – resultados no definidos.

A

	Initial				Total			
	Richness		Diversity		Richness		Diversity	
	$n = 11$	$n = 35$	$n = 11$	$n = 35$	$n = 47$	$n = 65$	$n = 47$	$n = 65$
MHS	–36.4	–0.6	1.1	39.3	0	–	67.1	–
TS	–77.4	–70.0	–67.8	–65.9	–76.3	–60.0	–59.2	–58.8
PFS	–70	–	–57.0	–	–	–	–	–

B

	Initial					Total			
	Richness		Diversity			Richness		Diversity	
	$S = 3$	$S = 10$	${}^1D = 2.0$	${}^1D = 2.4$	${}^1D = 5.5$	$S = 4$	$S = 10$	${}^1D = 2.0$	${}^1D = 4.8$
MHS vs. TS	8.8	–	10.5	–	–	10.8	–	7.0	–
MHS vs. PFS	2.8	–	2.0	3.7	–	–	–	–	–
MHS vs. (Sum)	1.3	2.3	1.5	1.3	7.5	1.5	2.4	1.0	12.6
TS vs. PFS	0.3	–	0.1	–	–	–	–	–	–
TS vs. (Sum)	0.1	–	0.1	–	–	0.1	–	0.1	–
PFS vs. (Sum)	0.5	–	0.8	0.4	–	–	–	–	–

$PAR_{35} = -0.6\%$) or non-existent (i.e., for the whole field work, with 47 individuals; table 2). The other two sampling methods presented negative biases (i.e., $PAR < 0$), with absolute values above 60% (table 2). As for species diversity, MHS displayed positive bias (e.g. for the first study period: $PAD_{35} = +39.3\%$), whereas the other two sampling methods showed negative biases. For all our field work, the absolute value of the bias corresponding to the diversity measure from MHS ($PAD_{47} = +67.1$) was slightly above that from TS ($PAD_{47} = -59.2\%$; table 2).

In all the comparisons between sampling methods, regardless of the richness or diversity values used in the comparisons, MHS was always the most efficient method to estimate both the number of species and the true species diversity (table 2). To achieve the maximum richness obtained during the first study period with TS or PFS (i.e., $S = 3$), MHS was between 1.3 (vs. all the methods combined) and

8.8 (vs. TS) times more efficient. More significantly, to achieve the total number of species recorded in our study (i.e., $S = 10$), MHS was 2.3 times more efficient than all methods combined. TS was the least efficient method to estimate both species richness and diversity (table 2).

Discussion

As biodiversity studies can not usually determine total richness in a local assemblage through exhaustive enumeration of all the species (but see González–Oreja et al., 2010), extrapolation (Colwell and Coddington, 1994; Gotelli and Colwell, 2001) or other techniques (Gotelli and Chao, 2013) are often needed. Our results show that the reptile assemblage in the study area was composed of 10 species; or, *sensu* Longino et al. (2002), that the set of species

really sampled by the methods used was composed of only 10 species. Moreover, all 10 species were recorded with only one sampling technique (i.e., microhabitat surveys), and with a small sampling effort. Our results also show that MHS was the best method (i.e., the least biased and the most efficient method) to determine the number of species.

In biodiversity studies with other animal groups, various authors have observed that sampling methods such as the MHS we used (e.g., the hand collection of ants and spiders) can not only be applied in settings where environmental conditions can exclude the use of other sampling methods but they can also be more efficient (King and Porter, 2005; Gotelli et al., 2011). Notwithstanding, sampling methods such as MHS and hand-collecting are influenced by the previous experience of the field worker (Longino et al., 2002; Ellison et al., 2007; Gotelli et al., 2011; see, also, Cardoso, 2009), which makes their standardization difficult (Blomberg and Shine, 2006; see also Mehrabi et al., 2014). For instance, expert field herpetologists could direct their attention, deliberately or not, to those microhabitats where rare species can be recorded. Inter-observer bias can be ruled out in our study as all the field work was completed by one observer (i.e., the first author of this study). Like many other authors (e.g. Peterson et al., 2004; Hutchens and DePerno, 2009; Fernández Badillo and Goyenechea–Mayer Goyenechea, 2010; Percino Daniel et al., 2013; Hidalgo Penninger, 2014), we also used transect surveys and pitfall-trap stations. On one hand, TS were strongly biased, even with large sampling efforts, and were the least efficient method. A possible reason for this negative result is that, during the TS, the observer was detected by the certain reptile species first, and not the other way round. This could help to explain the low accumulated total richness obtained with this method. On the other hand, the accumulation of species by PFS did not approach the asymptote even with the highest number of captured individuals. It would thus be interesting to replicate our study and evaluate the performance of this method with larger sampling efforts. Consequently, at least to determine the number of reptile species in the dry scrubland we studied, intensive searching for the target species in those sites that are frequently used as refuges would suffice.

However, in line with the limitation previously observed regarding estimation of the total richness in the study area (sensu Longino et al., 2002), it can not be ruled out that other sampling methods could have detected new reptile species. There is usually no sampling method that allows all the species in a local assemblage to be captured (because of the detection bias; Yoccoz et al., 2001), and different sets of species can be under-represented or over-sampled in the samples obtained by contrasting methods (Gotelli and Colwell, 2001). Still, the number of species in our reptile assemblage is similar to the species richness other authors have documented in thorny scrublands (six or seven species: Fernández Badillo and Goyenechea–Mayer Goyenechea, 2010) and dry shrublands (nine species: Vite Silva et al., 2010) from other Mexican regions (see also Ramírez Bautista et al., 2010).

Now, we will answer the question we raised concerning whether the best method to determine the number of species can simultaneously be the best method to estimate the true diversity of the local assemblage. This was clearly not the case in our study. If the species diversity accumulated by pooling all the sampling methods (i.e., the structured inventory) were the best available estimation of the actual diversity (as has been considered by previous authors: Walther and Moore, 2005; Ellison et al., 2007), then the best method to estimate species richness (i.e., MHS) was also a biased method (as were the other sampling techniques) that overestimated the actual diversity value as much as other methods underestimated it. Our finding does not support the suggestion by Steiner et al. (2005; cited by Gotelli et al., 2011) that, to compare between sites or habitat diversity measures in animal assemblages (in their case, ants), it can be better to use a single collecting method. Presumably, a structured inventory (even with an unbalanced design; Cardoso, 2009) will expose a more accurate image of the studied assemblage; therefore, the best sampling method to study species richness may not be the best method to study species diversity. Although combining different collecting methods may increase the budgets of time, field-work and money needed for the study (Gotelli et al., 2011), not to mention the need to consider the specific biases of each method (Gotelli et al., 2011), the structured inventory can be the most convenient way to study species diversity. Other studies with structured inventories usually observe that some sampling methods can find sets of unique species that are not recorded using other methods (King and Porter, 2005; Hutchens and DePerno, 2009; Gotelli et al., 2011). In our case study, MHS (which was also a biased method to estimate species diversity) could have overestimated the abundance of those species linked to bodies of water, like *K. integrum* and *T. cyrtopsis*. Moreover, because of its arboreal habits, the abundance of the Oaxacan oak anole (*Anolis quercorum*) could have been underestimated by the same method. If this were true, it would be appropriate to combine diverse sampling methods at the same time and obtain the species inventory with the highest possible diversity. Otherwise, estimates of species diversity could be misleading.

Finally, our study brings into question the utility of comparing estimates of species diversity obtained with different methods. Whereas it is true that the best method to estimate the number of species in a local assemblage cannot yield a higher value than the true richness, and therefore comparisons of species richness obtained with contrasting sampling methods can result in reasonable values, this is not true for the estimation of species diversity. In fact, even the best method to estimate species diversity can over- or under-estimate the true value. Therefore, not knowing the corresponding bias (or the accuracy) of the sampling methods involved in the comparison can render useless comparisons of diversity estimates between methods.

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