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Effect of trapping methods on the estimation of alpha diversity of a phlebotomine sandfly assemblage in southern Mexico

J. J. RODRÍGUEZ-ROJAS and E. A. REBOLLAR-TÉLLEZ

Laboratorio de Entomología Médica, Departamento de Zoología de Invertebrados, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Mexico

Abstract. The aims of the study were to (a) investigate the effect of trapping methods on alpha diversity; and (b) enhance the knowledge of the sandfly assemblage in the state of Quintana Roo. Field work was undertaken in a tropical forest of southern Mexico from August 2013 to July 2014. Sampling was conducted monthly during three consecutive nights. For each trapping night, 12 different types of trap were operated from 18.00 to 24.00 hours in four transects. Measures of alpha community diversity were based on the quantification of the number of species (Chao 2, Jackknife 2, Clench's equation, Margalef's index) and the community structure, as well as the dominance (Simpson and Berger-Parker indexes) and evenness (Shannon's entropy index, true diversity of the Jost and Pielou index). With a total sampling effort of 1728 night-traps, 16 101 phlebotomine sandflies were collected; they represented two genera and 13 species. Diversity estimates of 100% (Chao 2 and Clench's equation) and 85% (Jackknife 2) of potential species in the study area were calculated. Shannon traps and CDC light traps indicated the largest number of species, but only Shannon traps showed the greatest abundance. This inventory of sandflies is an important activity to enhance our knowledge of sandfly assemblages and guilds. The ultimate goal of studying alpha diversity in sandflies would be to have a better understanding of the population dynamics and all complex networks of interactions that may, in turn, be associated with the epidemiology of the disease.

Key words. *Leishmania*, alpha diversity, Cutaneous leishmaniasis, Phlebotominae, trap efficiency, Mexico.

Introduction

Knowledge of all biotic components of a particular place must be understood in its true dimension so that the report of the species present in a given community is of fundamental value in terms of biodiversity, biogeography and conservation biology (Speight *et al.*, 2008; Magurran & McGill, 2011). The most commonly used aspect of biodiversity is species richness, although this sole parameter does not account for other components of diversity. In particular, alpha diversity is represented by the species richness within a given community; and several quantitative methods have been developed and these methods are based on the number of species, abundances as well as the dominance and evenness (Moreno, 2001; Magurran, 2004; Magurran & McGill, 2011). To estimate biodiversity in a given time and site, reliable biological information is required and to achieve this, it is necessary to standardize sampling efforts. Therefore, a systematic sampling effort would permit to obtain valid representative inventories.

Studies on insects of medical importance are numerous and contribute to the knowledge on a variety of fields such as taxonomy, ecology, vector-incrimination, etc. One group of dipterian flies of medical importance are phlebotomine sandflies

Correspondence: Dr Eduardo A. Rebollar-Téllez, Departamento de Zoología de Invertebrados, Universidad Autónoma de Nuevo León, Avenida Universidad s/n, Ciudad Universitaria, San Nicolás de los Garza, Nuevo León C. P. 66455, Mexico. Tel.: +52 (81) 8332 4714; Fax: +52(81) 8332 1453; E-mail: eduardo.rebollartl@uanl.edu.mx

(Psychodidae: Phlebotominae) which they are the vectors of several pathogens such as *Leishmania* (Ross) spp., *Bartonella bacilliformis* (Barton) and *Phlebovirus*, *Vesiculovirus*, among others (Tesh, 1988; Killick-Kendrick, 1990; Comer & Tesh, 1991; Alexander, 1995). Phlebotomine sandflies species inventories can be best described when several collecting methods are incorporated in field studies, because it is known that some phlebotomine sandflies species are more attracted to particular traps than others (Maroli *et al.*, 1997; Alexander, 2000). Adult phlebotomine sandfly species have been collected using a variety of traps which are based on several behaviours such as phototaxis or using chemical clues associated with blood-seeking, egg-laying, sexual pheromones as well as interception traps (see the reviews of Maroli *et al.*, 1997; and Alexander, 2000).

In Mexico, taxonomical works can be traced back to 1934 when the first species were reported in the country (Galliard, 1934a, 1934b). Since then, the number of species and knowledge of geographical distribution has increased substantially (Vargas & Díaz-Nájera, 1953; Ibáñez-Bernal, 2000a; Ibáñez-Bernal *et al.*, 2015). Nonetheless, field studies describing the diversity of the phlebotomine sandfly assemblage are currently scant in the country. To date, Mexico species richness is represented by 48 extant species and 2 extinct species, although the number species may be underrepresented as many areas of the country remained unexplored (Vargas & Díaz-Nájera, 1953; Ibáñez-Bernal, 2000a, 2000b; Ibáñez-Bernal *et al.*, 2004, 2006, 2010, 2011, 2013, 2015; Rebollar-Téllez *et al.*, 2004; Godínez-Álvarez & Ibáñez-Bernal, 2010; May-Uc *et al.*, 2011). Sandfly species in Mexico have been collected mainly with three types of traps: Shannon traps (Shannon, 1939; Pérez *et al.*, 1988), CDC light traps (Center for Disease Control and Prevention) (Sudia & Chamberlain, 1962) and Disney traps (Disney, 1966), and to a lesser extent Malaise traps (Malaise, 1937) and Magoon traps (Magoon, 1935). Regardless of the study sites, one common observation in field studies conducted in Mexico is that species composition and abundances vary in accordance with trapping methods employed (e.g. Rebollar-Téllez *et al.*, 2005). If the collection methods influence species composition and abundances, then the estimators of diversity could be unrealistic or biased if only one sampling method is used.

So far, the only study conducted in Mexico to assess the diversity of phlebotomine sandflies quantitatively has been that of May-Uc *et al.* (2011). This study was carried out in 18 different sites belonging to three different zones (north, central and south regions) and catches were mainly undertaken within the same period (January–April). Even though the study of May-Uc *et al.* (2011) shed light on the diversity structure of sandflies in the state of Quintana Roo, other questions remained unanswered. For instance, the diversity structure over a longer period (for instance an annual cycle) has not been documented. Furthermore, we consider that an important issue was to estimate alpha diversity parameters when using and evaluating separately several trapping methods. With this in mind, the aims of the present study were to (a) investigate the effect of trapping methods on the quantification of alpha diversity; and (b) enhance the knowledge of the sandfly assemblage in the state of Quintana Roo.

Fig. 1. Depiction of an experimental transect. Sampling period was from August 2013 to July 2014 in Limones, Bacalar, Quintana Roo, Mexico. [Colour figure can be viewed at wileyonlinelibrary.com].

Materials and methods

Study site

The study was conducted in the locality of Limones, Bacalar, Quintana Roo, Mexico (18°59′26″ N, 088°09′04″ W; 21 m a.s.l.). Field work was carried out in a tropical forest that biogeographically belongs to the Neotropical region and Yucatan Peninsula as the biotic province (Barrera-Marin, 1962; Morrone, 2001) (for a more detailed description of the study site, see Rodríguez-Rojas *et al.*, 2016).

Phlebotomine sandfly collecting, processing and identification

Field work was carried from August 2013 to July 2014. A sampling of sandflies was conducted monthly during three consecutive nights. On each night, 12 different variations of trapping methods were employed using four replicates per trap. To minimize biases due to sampling transects, a block-design was used to randomize the position of each trap along the experimental transects. The experimental transects were composed of narrow paths that were carefully measured. Vegetation in the area comprises a sub-tropical forest with a dense understory growth (Fig. 1). Each trap type on transects was considered as a different treatment, and their location on each experimental unit was identified and labelled using different colour flagging tapes (Forestry Suppliers Inc., Jackson, MS, U.S.A.). Trapping methods were: 1.CDC light trap with blue LED (CDC-B), 2.-CDC light trap with white LED (CDC-W), 3.-CDC light trap with incandescent bulb (CDC-I), 4.-CDC light trap with red LED (CDC-R), 5.-CDC light trap with green LED (CDC-G), 6.-Disney trap, 7.-Disney trap with white LED (Disney-W), both types of Disney traps were baited with BALB/c mice, 8.-Sticky panels, 9.-Sticky panels with white LED (Sticky-W), 10.-Delta-like trap, 11.-Delta-like trap with white LED (Delta-W) (see more of the traps description in Rodríguez-Rojas *et al.*, 2016) and 12.-Shannon trap, which consists of a rectangular white tent

($2.5 \times 1.6 \times 1.5$ m) and its main attraction were the host-derived volatiles emanating through the perspiration and the carbon dioxide exhaled by the collectors ($n = 2$ per trap). All individuals acting as baits inside Shannon traps wore protective clothing to minimize exposure to infectious bites of female sandflies. Volunteers in the Shannon traps were trained to collect all sandflies within the trap using a mouth-aspirator and flies were placed in vials labelled by date, trap, transect and trap location. All traps were operated from 18.00 to 24.00 hours and were separated from other traps by at least 25 m. This period was selected based on previous studies in which activity of sandflies had been reported (Biagi *et al.*, 1966; Rebollar-Téllez *et al.*, 1996). Traps were hung at a height of 1.5 m, except for Shannon and Disney's traps which were hung at approximately 40 cm above the ground.

Collected sandflies from each trap were placed in 8-mL plastic vials containing 70% ethanol and were kept in a −20 °C freezer until they were processed. Each specimen was prepared for permanent slide-mounting according to the technique recommended by Young & Perkins (1984) and later modified by Ibáñez-Bernal (1999), and Euparal® (BioQuip Products, Rancho Dominguez, CA, U.S.A.) was used as mounting medium. Identification was supported using different morphological structures according to the keys of Young & Duncan (1994), as well as Ibáñez-Bernal (2005a, 2005b). Species reported herein follow the classic nomenclature system of Lewis *et al.* (1977). Voucher specimens are held in the entomological collection of Medical Entomology Laboratory, Faculty of Biological Sciences at the Autonomous University of Nuevo Leon, Mexico.

Statistical analysis

To determine alpha diversity estimates for each trapping method, an Excel® database was prepared; in which we organised all information about species, abundances, dates and trapping methods. Later, the total richness per trap (S) was quantified, as well as the number of singletons or doubletons in the collection. To estimate the total richness and the relative richness per trapping method, Margalef's species richness index was calculated (D_{Mg}) (Margalef, 1958; Moreno, 2001; Magurran, 2004; Magurran & McGill, 2011).

To assess the number of species that should be present in the area as a function of the trapping effort, several models were calculated. The accumulation species models were: (a) Clench's equation (Clench, 1979) which employs non-linear regressions with Simplex and Quasi Newton logarithms in STATISTICA software, version 8.0 (StatSoft Inc., Tulsa, OK, U.S.A.). Accumulation species curves per each trapping method were calculated to estimate the relationship between the parameters a/b (Clench, 1979; Soberón & Llorente, 1993; Jiménez-Valverde & Hortal, 2003). Other non-parametric models such as: (b) Chao 2 (Chao, 1987) and (c) second-order Jackknife's equation (Jackknife 2) (Quenouille, 1956; Smith & van Belle, 1984), were also calculated to estimate the expected number of species per each trapping method as a function of the sampling effort (Colwell & Coddington, 1994; Moreno, 2001). To estimate each parameter, the software program was set at 100 randomizations of the dataset before analysis in EstimateS version 9.1.0 (Colwell, 2013).

Other estimations were carried out to analyse the assemblage structure in relation with the proportional abundances. Shannon's entropy function (H′) (Shannon, 1948) was computed taking into account the number of species per trapping method and their relative proportion of individuals. The so-called 'true diversity' index (^{q}D) was also quantified. The true diversity index is positively correlated with the effective number of species. For this reason, the effective number of species of order 1 (^{1}D) per each trap (Jost, 2006, 2007) was measured. Whereas, eveness was estimated using Pielou's index (J′) (Pielou, 1975). Basically, this parameter measures the uniformity of all individuals between all taxa. Simpson's dominance index (λ) (Simpson, 1949) was also calculated to identify those species whose contribution out-numbers the other species. Berger-Parker's dominance index (d) (Berger & Parker, 1970) was calculated to quantify the actual proportion of the most common species among the sandfly assemblage (Moreno, 2001; Magurran & McGill, 2011).

Shannon's entropy index among all trapping methods were compared statistically using a modified Student's t-test (Hutcheson, 1970; Zar, 1999), and likewise the probability values were obtained from the Simpson index (Simpson, 1949; Brower *et al.*, 1998) with PAST program version 3.11 (Hammer *et al.*, 2001), and $P \leq 0.05$ value was considered to be significant. To analyse the patterns of species-abundances per each trapping method, standardized rank-abundance plots were constructed to compare species richness (number of data points) (X-axis) and their relative abundances (Y-axis) (Whittaker, 1965; Magurran, 2004). Finally, to evaluate the most abundant species of phlebotomine sandflies, 'Standardized Index of Species Abundance' (SISA) was utilized (Roberts & Hsi, 1979). This index is a ranking procedure to compensate abundances and trapping methods. The SISA index values range from 0 to 1, and a valued close to 1 correspond to the most abundant species.

Results

Assemblage structure

Using 12 different traps over a year, a total sampling effort of 1728 night-traps was made, and the capture effort per month was 144 night-traps. In the entire annual sampling period, a total of 16 101 phlebotomine sandfly specimens belonging to 2 genera and 13 species were collected. Overall, the most abundant species regardless of trapping method were: *Lutzomyia cruciata* (Coquillett) (42.33%; SISA = 0.65), *Lutzomyia shannoni* (Dyar) (32.68%; SISA = 0.44), *Brumptomyia mesai* (Sherlock) (9.75%; SISA = 0.48) and *Lutzomyia ovallesi* (Ortíz) (9.03%; SISA = 0.17). Other less abundant species were: *Lutzomyia steatopyga* (Fairchild and Hertig) (1.81%), *Lutzomyia deleoni* (Fairchild and Hertig) (1.76%), *Lutzomyia olmeca olmeca* (Vargas and Díaz-Nájera) (1.23%), *Lutzomyia carpenteri* (Fairchild and Hertig) (0.34%), *Lutzomyia cratifer* (Fairchild and Hertig) (0.30%), *Lutzomyia cayennensis maciasi* (Fairchild and Hertig) (0.13%), *Lutzomyia trinidadensis* (Newstead) (0.06%), *Lutzomyia permira* (Fairchild and Hertig) (0.01%) and *Lutzomyia manciola* Ibáñez-Bernal (0.01%) (Table 1). The latter

Table 1. Species richness and absolute abundances of phlebotomine sandfly species collected over a year period using 12 different types of traps.

sppCode	Species	CDC-W	CDC-B	CDC-I	CDC-R	CDC-G	Disney	Disney-W	Sticky	Sticky-W	Delta	Delta-W	Shannon	Total (%)	SISA
mes	*Brumptomyia mesai*	24	26	38	39	13	0	2	0	0	0	0	1428	1570 (9.75)	0.48
car	*Lutzomyia carpenter*	21	16	12	1	4	0	0	0	0	0	0	1	55 (0.34)	0.19
mac	*L. cayennensis maciasi*	5	6	0	1	7	0	0	0	0	0	0	2	21 (0.13)	0.12
cra	*L. cretifer*	0	0	1	0	0	0	0	0	0	0	0	47	48 (0.30)	0.05
cru	*L. cruciata*	20	23	28	9	11	4	2	2	2	0	5	6710	6816 (42.33)	0.65
del	*L. deleoni*	45	28	71	29	45	0	0	0	0	0	3	63	284 (1.76)	0.50
man	*L. manciola*	0	0	0	0	0	0	0	0	0	0	0	2	2 (0.01)	0.01
olm	*L. olmeca olmeca*	13	9	14	10	15	61	9	0	1	1	1	64	198 (1.23)	0.59
ova	*L. ovallesi*	4	2	11	3	0	0	0	0	0	0	0	1434	1454 (9.03)	0.17
per	*L. permira*	0	1	1	0	0	0	0	0	0	0	0	0	2 (0.01)	0.02
sha	*L. shannoni*	7	7	21	16	8	1	0	0	0	0	2	5287	5349 (33.22)	0.44
ste	*L. steatopyga*	38	40	88	38	49	0	1	1	0	0	1	36	292 (1.81)	0.34
tri	*L. trinidadensis*	1	1	1	0	3	0	1	0	0	0	0	3	10 (0.06)	0.13
	Abundances	178	159	286	146	155	66	15	3	3	1	12	15077	16101 (100)	
	Species richness (S)	10	11	11	9	9	3	5	2	2	1	5	12	13	

Data also include the relative percentage of each species and their corresponding ranking order according to SISA. Sampling period was from August 2013 to July 2014 in Limones, Bacalar, Quintana Roo, Mexico.

Table 2. Summary of all alpha diversity estimators of the phlebotomine sandfly assemblage according to each trapping method.

Estimators	CDC-W	CDC-B	CDC-I	CDC-R	CDC-G	Disney	Disney-W	Sticky	Sticky-W	Delta	Delta-W	Shannon	All traps
Abundance	178	159	286	146	155	66	15	3	3	1	12	15077	16101
Species richness (S)	10	11	11	9	9	3	5	2	2	1	5	12	13
Singletons	1	2	3	2	0	1	2	1	1	1	2	1	0
Doubletons	0	1	0	0	0	0	2	1	1	0	1	2	2
Margalef (D_{Mg})	1.74	1.97	1.77	1.61	1.59	0.48	1.48	0.91	0.91	0.00	1.61	1.14	1.24
Shannon entropy (H')	1.98	2.00	1.86	1.77	1.81	0.31	1.21	0.64	0.64	0.00	1.42	1.26	1.42
True diversity (^1D)	7.24	7.39	6.42	5.87	6.11	1.36	3.35	1.90	1.90	1.00	4.14	3.53	4.14
Equitativity (J')	0.86	0.84	0.78	0.81	0.82	0.28	0.75	0.92	0.92	0.00	0.88	0.51	0.56
Simpson (λ)	0.16	0.16	0.20	0.20	0.21	0.86	0.40	0.56	0.56	1.00	0.28	0.34	0.31
Berger-Parker (d)	0.25	0.25	0.31	0.27	0.31	0.92	0.60	0.67	0.67	1.00	0.42	0.45	0.42

Study period was from August 2013 to July 2014 in Limones, Bacalar, Quintana Roo, Mexico.

species were poorly represented in the collection, and represented together only 5.66% of the total catch. Two doubletons were identified: *L. permira* and *L. manciola* (Table 2, Fig. 3).

According to Clench's equation, the total number of caught species (13 species) was equal to the expected number of present species (a/b = 13.14 species). Considering all data pooled together; phlebotomine sandfly assemblage reached 85.92% of the total expected species by the 24th sampling night. With Chao's 2 estimator, the 13 observed species in the study represented 100% of the total expected number of species. Whereas, Jackknife's 2 estimated only 85% of the species were detected (Table 3, Fig. 1).

Alpha diversity estimates, such as Shannon's entropy index and the true diversity, produced values of $H' = 1.42$ and $^1D = 4.14$ effective numbers of species, respectively. Simpson's dominance index yielded $\lambda = 0.31$, whereas Berger-Parker's species dominance index was $d = 0.42$. Finally, evenness index gave an estimate of $J' = 0.56$ (Table 2).

Species richness per trap

When sampling effort was analysed by each trapping method, each type of trap was used for 144 night-traps. Shannon traps collected a total of 12 species, followed by the trap types CDC-B and CDC-I with 11 species every trap. In contrast, the trap types Delta, Sticky, Sticky-W and Disney collected the lowest number of species (see Table 2, Fig. 3).

Two species, *L. cruciata* and *L. olmeca olmeca*, were frequently sampled in 11 of the 12 trap types but *L. cruciata* was not captured in the Delta trap, and *L. olmeca olmeca* was not captured in a Sticky trap. Rare species were represented by *L. manciola* in Shannon traps, as well as *L. cratifer* and *L. permira* that were caught only in two types of traps (Table 1, Fig. 3). Singletons (n = 3) were mainly found in the CDC-I traps, whereas, doubletons ($n = 2$) were mainly seen in Disney-W and Shannon traps (Table 2). When calculating Margalef's species richness index, it was observed that the trap type CDC-B had the highest value (1.97), followed by CDC-I (1.77), CDC-W (1.74), CDC-R (1.61), Delta-W (1.61) and CDC-G (1.58) (Table 2).

Using Clench's equation for each type of trap, CDC-B and Shannon traps had the highest expected number of species, with 11.95 and 11.89 species, respectively, followed by CDC-I (a/b = 11.39), CDC-W (a/b = 10.86), CDC-R (a/b = 9.52) and CDC-G traps (a/b = 10) (Table 3, Fig. 2). According to Chao's 2 model, the highest expected number of species was estimated for CDC-I and Shannon traps; with 12.49 and 12 species, respectively. While the Jackknife 2 estimator, the predicted number of species was15.96 species for the trap type

Table 3. Observed species richness (S obs) and results of three species richness estimators for each trap.

		Clench				Chao 2				Jackknife 2	
Traps	S obs	a/b	r^2	Slope	%	S est	IC (95%)	SD	%	S est	%
CDC-W	10	10.86	0.999	0.006	84	10.00	10.07–11.10	0.25	77	11.00	85
CDC-B	11	11.95	0.998	0.007	92	11.50	11.03–19.21	1.29	88	13.98	108
CDC-I	11	11.39	0.981	0.006	88	12.49	11.15–25.99	2.58	96	15.96	123
CDC-R	9	9.52	0.995	0.005	73	9.50	9.03–17.21	1.29	73	11.98	92
CDC-G	9	10.00	0.998	0.005	77	9.00	9.00–10.07	0.43	69	8.02	62
Disney	3	2.18	0.998	0.001	17	2.00	2.00–2.28	0.13	15	2.00	15
Disney-W	5	8.86	0.997	0.015	68	6.49	5.15–19.99	2.58	50	9.96	77
Sticky	2	5.53	0.999	0.008	43	2.00	2.02–3.32	0.25	15	3.00	23
Sticky-W	2	5.53	0.999	0.008	43	2.00	2.02–3.32	0.25	15	3.00	23
Delta	1	−23.89	0.999	0.007	−184	1.00	1.00–3.07	0.48	8	2.98	23
Delta-W	5	9.29	0.999	0.016	71	5.50	5.03–13.21	1.29	42	9.98	77
Shannon	12	11.89	0.954	0.002	91	12.00	12.39–13.07	0.17	92	12.02	92
All traps	13	13.00	0.966	0.001	100	13.00	13.00–14.24	0.48	100	11.04	85

Non-parametric estimators Chao 2 and Jackknife 2 for the total number of expected phlebotomine sandfly species (S est). Clench's equation data are also included for each trapping method, where a/b is the asymptote (S est). Sampling period was from August 2013 to July 2014 in Limones, Bacalar, Quintana Roo, Mexico.

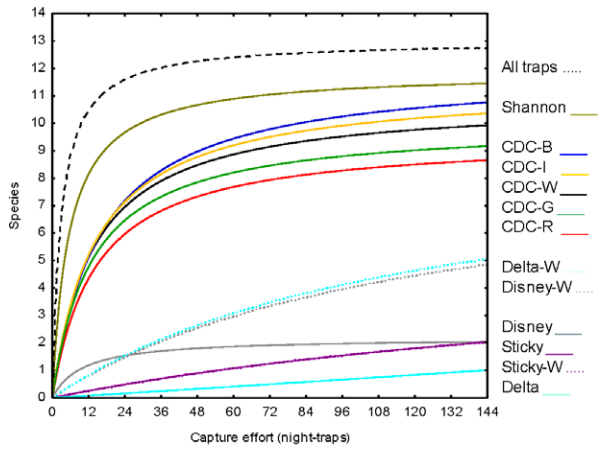

Fig. 2. Species accumulation curves for the observed number of species (S obs), the expected number of species according to Clench's (1979) equation. As a reference in the plot the total accumulation curve when all data were pooled together is included. The sampling period was from August 2013 to July 2014 in Limones, Bacalar, Quintana Roo, Mexico. [Colour figure can be viewed at wileyonlinelibrary.com].

CDC-I and 13.98 species for the trap type CDC-B (Table 3, Fig. 2).

Abundance per trap

Considering the relative abundance of each trapping method, Shannon traps had an overwhelming majority over the rest of the 11 types of traps. Shannon traps collected 15 077 sandfly specimens representing 93.64% of the total, which were dominated by four sandfly species: *L. cruciata* (44.50%), *L. shannoni* (35%), *L. ovallesi* (9.51%) and *B. mesai* (9.47%) (Table 1). Together, CDC-I traps and CDC traps fitted with LED colour lights collected 5.73% of the total catch. CDC-I traps collected 286 specimens and the most abundant species were: *L. steatopyga* (30.77%) and *L. deleoni* (24.83%), whereas in all CDC types with LED lights 638 specimens were collected and the most abundant species were: *L. steatopyga* (25.86%), *L. deleoni* (23%) and *B. mesai* (15.99%) (Table 1, Fig. 3).

Heterogeneity values measured by Shannon's entropy index showed that the estimates were obtained for CDC-B and CDC-W traps; $H' = 2$, $H' = 1.98$. Both trap types also obtained the highest estimates for 'true diversity'; $^1D = 7.39$ and $^1D = 7.24$, respectively for the effective numbers of species. In contrast, the lowest estimates were seen in Delta-like traps ($H' = 0$; $^1D = 1$) and Disney traps ($H' = 0.31$; $^1D = 1.36$); although the highest dominance index was calculated for Disney traps ($\lambda = 0.86$; $d = 0.92$) (Table 2). However, the highest evenness estimates were calculated for Sticky ($J' = 0.92$) and Sticky-W ($J' = 0.92$) traps. A matrix of significant and non-significant values of modified t-test of Hutcheson for Shannon's entropy index as well as Simpson's index is presented in Table 4.

Discussion

The present study, assessing the phlebotomine sandfly assemblage using 12 different traps, provided a species richness of 13 phlebotomine sandfly species, representing 26% of all Phlebotominae species reported so far in Mexico (Vargas & Díaz-Nájera, 1953; Ibáñez-Bernal, 2000a, 2000b; Ibáñez-Bernal *et al.*, 2004; 2006, 2013, 2015; Godínez-Álvarez & Ibáñez-Bernal, 2010; Ibáñez-Bernal *et al.*, 2011), 50% in Yucatan Peninsula (Rebollar-Téllez *et al.*, 2004, 2005, 2006; Ibáñez-Bernal *et al.*, 2010; May-Uc *et al.*, 2011) and 54% of species of Quintana Roo (Ibáñez-Bernal *et al.*, 2010; May-Uc *et al.*, 2011). Although 12 of the species collected were previously known in Mexico, *Lutzomyia manciola* (2♀) represents a new record for the country but was described by Ibáñez-Bernal (2001) in Belize.

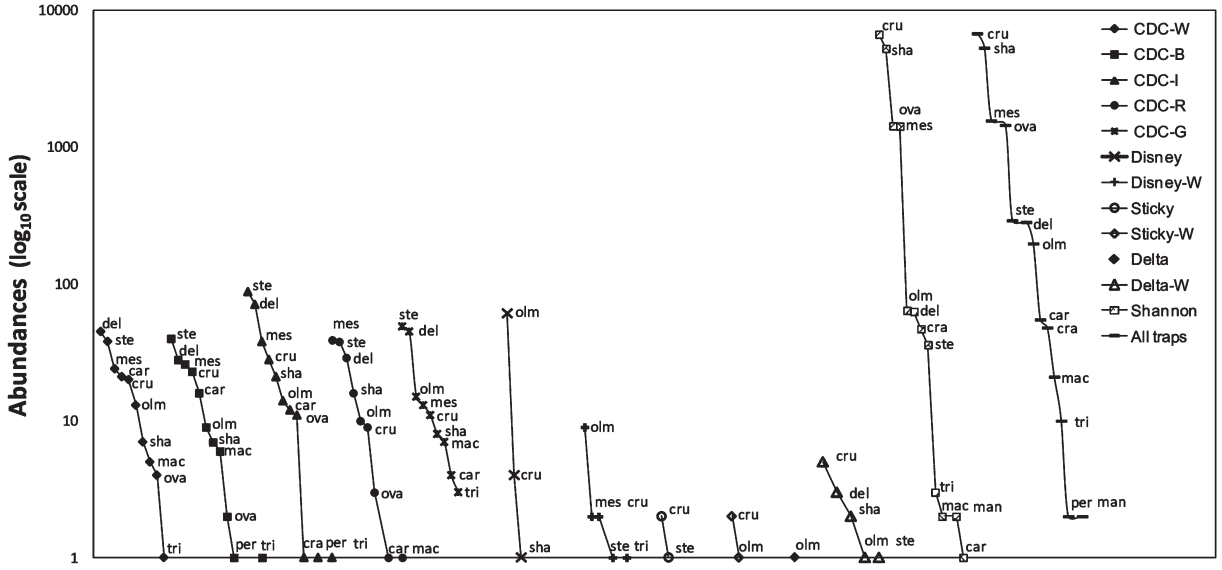

Fig. 3. Rank-abundance curves for all phlebotomine sandfly species collected with 12 different types of traps. Sampling period was from August 2013 to July 2014 in Limones, Bacalar, Quintana Roo, Mexico. Species codes are included in Table 1.

According to the estimates of species, a capture effort of 1758 night-traps was sufficient to obtain a representative inventory of phlebotomine sandfly species in the area: the accumulation curve reached the asymptote at 114 night-traps, representing at this point 99.30% of total species captured. Jiménez-Valverde & Hortal (2003) mentioned that the higher the sampling effort, the greater the number of species collected. Thus, theoretically, when the calculated slope drops to zero it corresponds to a representative inventory and high reliability. In the present study, the slope in Clench's equation was 0.001 in the total of all the traps.

There was significant variation between the number of species and numbers of sandflies caught between each trapping method. Shannon traps were by far the most efficient sampling method in this tropical forest. Similar results were obtained in several studies in the Mexican southeastern states of Campeche (Rebollar-Téllez *et al.*, 2005; Pech-May *et al.*, 2010, 2016), Yucatan (Rebollar-Téllez *et al.*, 2006), Quintana Roo (Cruz-Ruiz *et al.*, 1994; Sánchez-García *et al.*, 2010; May-Uc *et al.*, 2011) and Chiapas (Pérez *et al.*, 2013). It is important to highlight that Shannon traps were primarily developed to collect sylvan mosquitoes using a horse as a bait during an epidemic of jungle yellow fever in Mato Grosso and Rio de Janeiro, Brazil (Shannon, 1939), and many years later, Pérez *et al.* (1988) made several modifications for its use for sandflies using humans as bait. Other studies have used Shannon traps as a method to capture sandflies in Peru (Pérez *et al.*, 1988; Pérez & Ogusuku, 1995), Colombia (Vivero *et al.*, 2010; Posada-López *et al.*, 2014), Brazil (Silveira *et al.*, 2002; Calvo-Alessi *et al.*, 2009) and Argentina (Córdoba-Lanús & Salomón, 2002; Salomón *et al.*, 2004).

Of the 12 species captured in Shannon traps, the most abundant species were *L. cruciata, L. shannoni* and *L. ovallesi*. The dominance of these three species may be due to their marked anthropophilic behaviour as observed in several previous studies (e.g. Biagi *et al.*, 1965; Rebollar-Téllez *et al.*, 2005; Pech-May *et al.*, 2010, 2016; Sánchez-García *et al.*, 2010; May-Uc *et al.*, 2011). Furthermore, these species, together with *L. olmeca olmeca*, have been incriminated as vectors of *Leishmania mexicana* (Biagi *et al.*, 1965; Rebollar-Téllez *et al.*, 1996, 1996, 1996; Pech-May *et al.*, 2010, 2016; Sánchez-García *et al.*, 2010). *Lutzomyia olmeca olmeca* was collected in Shannon traps, Disney traps and CDC light traps, with similar abundances between these three traps, but dominated collections in Disney traps. In previous studies conducted in Mexico (Rebollar-Téllez *et al.*, 2005; Sánchez-García *et al.*, 2010), Panama (Christensen & Herrer, 1973), and Belize (Disney, 1968), *L. olmeca olmeca* is highly rodentophilic. However, it was also observed in Mexico to be relatively anthropophilic (Biagi *et al.*, 1965; Rebollar-Téllez *et al.*, 1996; Pech-May *et al.*, 2010; Sánchez-García *et al.*, 2010) and had limited attraction to light (Pech-May *et al.*, 2010; Rebollar-Téllez *et al.*, 1996). In addition, *L. olmeca olmeca* was the first species to be fully incriminated as a vector of *Leishmania mexicana* in Mexico, particularly in the state of Quintana Roo (Biagi *et al.*, 1965).

Shannon traps also captured a greater number of specimens of *B. mesai*, and *L. cratifer*, and the second species was exclusive to this trap type. Specimens of *B. mesai* were perhaps attracted by the light of the torches used in the trap, as observed in a previous study (Rebollar-Téllez *et al.*, 2005). However, although collections of *L. cratifer* were similar to the findings of Ibáñez-Bernal *et al.* (2004) and Pérez *et al.* (2013), very little is known regarding their anthropophilic behaviour. Other species that were caught in low abundances in Shannon traps were *L. carpenteri, L. deleoni* and *L. steatopyga*. The three species were shown to be highly attracted to CDC light traps, as shown in several studies of southern Mexico (Rebollar-Téllez *et al.*, 1996, 2005). The only species collected elsewhere in the study region but not by Shannon traps was *L. permira*, and perhaps this species can be considered rare (doubletons) although it has been

Table 4. Matrix of *P*-values associated with Shannon's entropy index and Simpson's index for each trapping method.

	CDC-W	CDC-B	CDC-I	CDC-R	CDC-G	Disney	Disney-W	Sticky	Sticky-W	Delta	Delta-W	Shannon
Shannon entropy index												
CDC-W		ns	ns	**	*	***	**	*	*	***	*	***
CDC-B	ns		*	**	*	***	**	*	*	***	*	***
CDC-I	*	*		ns	ns	***	*	*	*	***	ns	***
CDC-R	*	*	ns		ns	***	*	*	*	***	ns	***
CDC-G	*	*	ns	ns		***	*	*	*	***	ns	***
Disney	***	***	***	***	***		**	ns	ns	**	**	***
Disney-W	ns	ns	ns	ns	ns	**		ns	ns	***	ns	ns
Sticky	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns
Sticky-W	ns	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns
Delta	–	–	–	–	–	–	–	–	–		***	***
Delta-W	ns	ns	ns	ns	ns	***	ns	ns	ns	–		ns
Shannon	***	***	***	***	***	***	ns	ns	ns	–	ns	
Simposon index												

$*P \leq 0.05$.
$**P \leq 0.01$.
$***P \leq 0.001$.
Sampling period was from August 2013 to July 2014 in Limones, Bacalar, Quintana Roo, Mexico. ns, not significant.

collected previously using CDC light traps (Rebollar-Téllez *et al.*, 2005).

The present study was conducted entirely on the ground level, and the vertical distribution of sandflies was not determined. Other studies carried out in tropical forest canopy have reported that some phlebotomine sandfly species may vary their relative composition and abundances at different heights (Thatcher, 1968; Williams, 1970; Chaniotis *et al.*, 1972; Christensen *et al.*, 1972). Phlebotominae sandfly species associated with mammal burrows, ant-nest refuges or particular ecotopes (e.g. tree trunks, holes under rocks, buttress roots and leaf litter) have been documented in the literature (Hanson, 1961; Thatcher & Hertig, 1966; Comer & Corn, 1991; Rebollar-Téllez *et al.*, 1996). However, the present study did not include this faunistic component, so it may well be possible that other species are also present in the area.

Rank-abundance plots were first proposed by Whittaker (1965) and are generally effective methods for illustrating changes through a succession of species in a particular site. In the present investigation, CDC light traps provided evidence that the sandfly assemblage is more even than for the other types of traps and thus more diverse. Moreover, Shannon traps collections showed the dominance of certain sandfly species and had an intermediate evenness. The representativeness of these two type traps is to a certain extent homogeneous regarding the number of phlebotomine sandfly species of the assemblage but exhibited heterogeneity in its abundances.

The main contribution of this study was to demonstrate that when using the same capture effort for each trap, a variation in species richness and abundances are detected and thereby affecting alpha diversity estimates. This variation may be influenced by the relative attractiveness of certain different baits or lures in each capture method to sandfly species. Based on the findings observed in this study, caution must be taken when reaching conclusions relating to the abundances and diversity estimators when field studies are conducted in a given area. As trap efficiency and relative species richness varies, it is important to have a clear idea of the research hypothesis and objectives of the study to select appropriate collection methods and field design. Finally, complete inventories of sandfly species assemblages are best assessed when multiple types of traps are used. The ultimate goal of studying alpha diversity in phlebotomine sandflies is to have a better understanding of the population dynamics and all complex networks of interactions that in turn may be associated with the epidemiology of diseases transmitted by female sandflies.

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