Acta Biológica Catarinense
2017 Jan-Jun;4(1):5-12

Different sampling intervals for bowl trapping survey of Aculeata (Hymenoptera) in a semidecidual seasonal forest fragment

Diferentes intervalos de amostragem no inventário de Aculeata (Hymenoptera) em um fragmento de floresta estacional semidecidual

Cleiton **MARGATTO**[1, 2] & Rodrigo Barbosa **GONÇALVES**[1]

ABSTRACT
Coloured bowl trapping is a passive sampling method which catches insects through a combination of interception and attraction. The method is commonly used for Aculeata, especially bees. The objective of the present study is to determine a reliable sampling interval for aculeates using bowl traps since an appropriate sampling interval is important to enable the collection of representative samples in Neotropics. For this purpose we installed eight bowl traps in a forest fragment in Southern Brazil, during one year, to compare the sampled diversity in twice and once-a-month sampling interval. We confirmed the general expectation that increasing the sample size results in a proportional greater sampling of the community. Different sampling intervals have a weak impact in rarefaction curves, as these do not reach the asymptote in any cases, and Shannon diversity index was considered statistically equal. Under our results, the twice-a-month interval was considered better than monthly sampling intervals only when we compare the extrapolation data (estimators and extrapolation curve) for Aculeata. The Shannon diversity index and the estimated species number (rarefaction) were considered the same among the treatments, so we cannot suggest a reliable sampling interval from these data.
Keywords: Apoidea; bees; ecology; sampling effort.

Recebido: 6 dez. 2016
Aceito: 5 mar. 2017

RESUMO
Pratos-armadilha coloridos são um método de amostragem de insetos que combina intercepção de voo e atração. O método é comumente utilizado para Aculeata, especialmente abelhas. O objetivo do presente estudo é determinar um intervalo de amostragem confiável para aculeados utilizando pratos-armadilha, uma vez que um intervalo apropriado se mostra importante para permitir a coleta de amostras representativas. Para tanto foram instaladas oito armadilhas em um fragmento florestal no sul do Brasil, durante um ano, para comparar a diversidade obtida em intervalos de uma vez e duas vezes ao mês. Confirmou-se a expectativa de que o aumento do número de amostras resulta em uma amostragem proporcionalmente maior. Diferentes intervalos tiveram impacto fraco nas curvas de rarefação; elas não chegaram à assíntota em nenhum caso, e o índice de diversidade de Shannon foi considerado estatisticamente o mesmo. De acordo com os resultados, o intervalo de duas vezes ao mês foi considerado melhor que uma vez ao mês apenas quando comparados os dados de extrapolação (estimadores de diversidade e curva de extrapolação) para Aculeata. O índice de Shannon e o número estimado de espécies (rarefação) foram considerados os mesmos entre os tratamentos, então não se pode indicar um intervalo confiável com base em tais dados.
Palavras-chave: Apoidea; abelhas; ecologia; esforço de coleta.

[1] Departamento de Zoologia, Universidade Federal do Paraná (UFPR), Avenida Coronel Francisco Heráclito dos Santos, s/n, Jardim das Américas, Cx. Postal 19020, CEP 81531-980, Curitiba, PR, Brasil.

[2] Autor para correspondência: cleiton.margatto@gmail.com.

INTRODUCTION

Documenting a region's species richness is of crucial importance for the conservation and management of biodiversity (COLWELL & CODDINGTON, 1994). Richness estimation, as other assemblage metrics, is strongly linked with sampling methodology and is influenced by the target group, habitat type and study goal (NIELSEN *et al*., 2011). The traditional sampling method for flying aculeates (non-Formicidae) is hand-netting, since trap-based methods are frequently viewed as less efficient (CANE *et al*., 2001; LAROCA & ORTH, 2002). While hand-netting is considered effective for representativeness this method does not ensure comparability, a desirable issue for monitoring programs (MARTINS *et al*., 2013) and conservation studies (e.g., GIBB & HOCHULI, 2002; CALVILLO *et al*., 2010; GONÇALVES *et al*., 2014).

Coloured bowl traps (also referred as pan or Moericke traps) is one of the most commonly-used techniques to sample aculeates, particularly bees (ABRAHAMCZYK *et al*., 2010). Bowl trapping is a passive sampling method, with no collector bias (LEONG & THORP, 1999), which catches insects through a combination of interception and attraction (VRDOLJAK & SAMWAYS, 2012). Bowl traps are also easily replicated (WESTPHAL *et al*., 2008) and allow sampling multiple transects simultaneously (KRUG & ALVES-DOS-SANTOS, 2008; DROEGE *et al*., 2010). Studies on bowl trap methodology have focused in different colour preferences (e.g., ABRAHAMCZYK *et al*., 2010; HENEBERG & BOGUSH, 2014; MOREIRA *et al*., 2016), and only one study compared diversity and richness under different sampling intervals. Banaszak *et al*. (2014) found more bee species using weekly sampling intervals during the flowering season in Poland, ensuring the collection of about 75% of estimated species number. However, studies in tropical regions with a more continuous and extended plant blooming pattern are completely lacking.

With the need of an unbiased method to sample local flying aculeate fauna for comparative studies, the objective of the present study is to determine a reliable sampling frequency of aculeates using bowl traps to enable the collection of representative samples. For this purpose, we compare Aculeata diversity statistics between twice-a-month (fortnightly) and once-a-month sampling intervals for assemblages in a fragment of Semidecidual Seasonal Forest, Southern Brazil.

MATERIAL AND METHODS

This study was conducted in a 4.65 ha forest fragment in the western portion of Paraná State (Brazil), around the UTM coordinates -24.292846S, -53.842143W. Originally, the area was entirely covered by Semidecidual Seasonal Forest, a formation typical of the inland vegetation of the Brazilian Atlantic Forest biome. Currently the fragment belong to the campus of Universidade Federal do Paraná inside Palotina municipality and is surrounded by campus buildings.

Sampling was carried out in the warmer period of the day, between 9 A.M. and 3 P.M., from June 2014 to May 2015, twice-a-month, for a total of twenty-three sampling days (June was sampled only once). In each sampling day eight bowl traps were arbitrarily installed on the field at the edge of the fragment. The sampling details were selected to be comparable to other studies in Palotina (GONÇALVES *et al*., 2014). We used plastic food bowls, blue and yellow, with 14.5 cm of diameter at the upper borders, 10 of diameter in the mid portion, and 6 cm in height, filled one third of its volume with diluted detergent-water solution. The bowl traps were placed on the ground, alternated by colour and spaced 10 m apart from each other. The sampled Apidae, Crabronidae, Pompilidae, and

Vespidae were sorted to species and are deposited in Entomological Collection "Pe. Jesus Santiago Moure" (DZUP), Departamento de Zoologia (UFPR, Curitiba, Brazil).

We created three analysis datasets, the first used all samples from the 23 sampling days and represented the twice-a-month sampling interval (twice) using data from 184 individual bowl trap samples (table 1). The second and third analysis datasets were monthly subsets of the whole data set. For the second dataset we randomly selected one sampling day by month (random) and for the third dataset we selected the warmer sampling day by month (warmer). For each dataset we calculated the following diversity statistics: observed richness (S), abundance (N), Shannon diversity index (H), Chao-1 and Jack-1 species estimators. The sampling intervals statistics were compared with randomization tests (bootstrapping and permutation) using the Compare Diversity Module. The analyses were carried out with PAST statistical software package (HAMMER *et al.*, 2001). Sample based interpolation (rarefaction) and extrapolation curves (COLWELL *et al.*, 2012) were estimated with EstimateS9.1, the confidence intervals were used to compare the curves. Apidae assemblages were also analyzed alone due to their high richness and abundance among the treatments. Sampling success was measured by the ratio of effective samples (those with more than 0 individuals) and total samples.

RESULTS

We collected 125 individuals of 39 species of Aculeata with most species (24) belonging to Apidae followed by seven Pompilidae, four Crabronidae and four Vespidae species (tables 1-2). The sampling success of pan trapping varied from 35% to 42% for all datasets and all taxa analyzed (table 1). From the 23 sampling days, two did not sample any Aculeata, September and November 2014 (table 2). The ratio of species and individuals by trap unit was about 0.6 and 1.8 for Aculeata and 0.35 and 1.4 for Apidae (table 1).

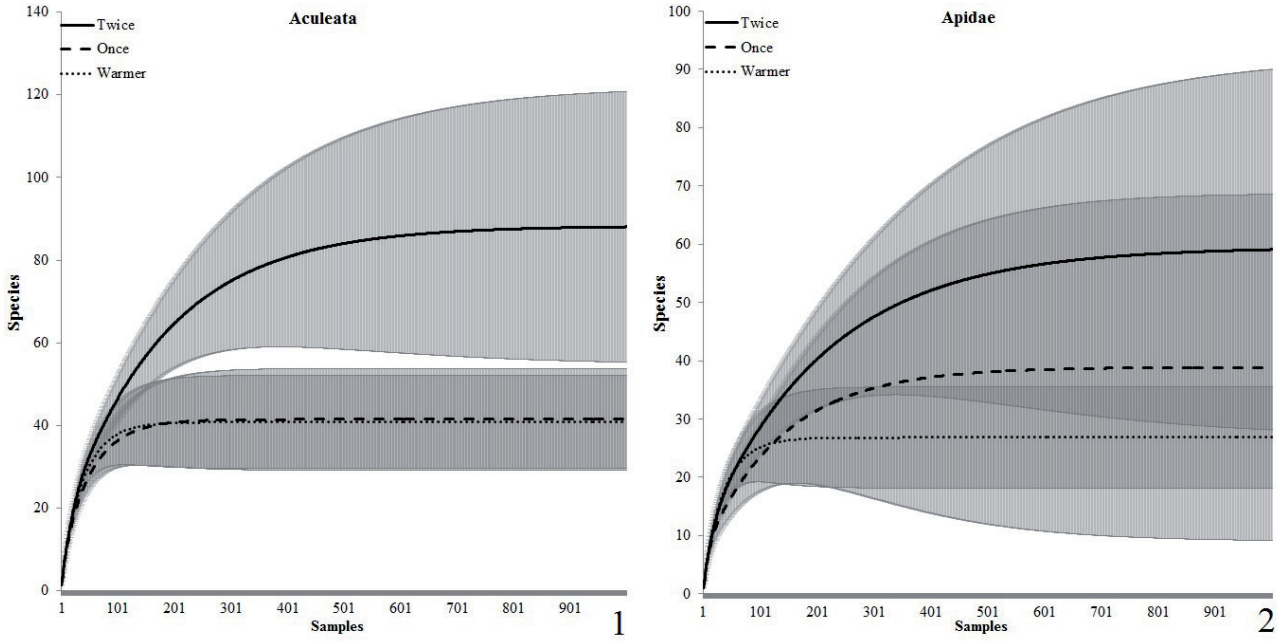
Table 1 – Diversity statistics for three sampling intervals, twice-a-month, once-a-month (random and warmer sampling days). Effective number of samples and % of total (under parenthesis), S = total species number and by trap (under parenthesis), N = total number of individuals and by trap (under parenthesis), H = Shannon diversity index (lower and upper 95% confidence interval), Chao-1 and Jack-1 (mean) estimators.

Family	Samples	S	N	H (l-u)	Chao-1	Jack-1
Aculeata						
Twice	71 (38%)	39 (0.55)	124 (1.74)	3.02 (2.76-3.19)	67.5	58.71
Once (random)	40 (42%)	25 (0.62)	66 (1.65)	2.77 (2.38-2.90)	34.43	37.67
Once (warmer)	34 (35%)	25 (0.73)	69 (2)	2.55 (2.00-2.62)	36.14	38.58
Apidae						
Twice	71 (38%)	24 (0.33)	94 (1.32)	2.40 (1.98-2.53)	37.75	35.83
Once (random)	40 (42%)	15 (0.37)	52 (1.3)	2.21 (1.73-2.34)	20	21.82
Once (warmer)	34 (35%)	17 (0.5)	57 (1.67)	2.11 (1.48-2.24)	22.6	25.73

The twice-a-month sampling interval presented higher values of observed richness and abundance which are statistically different from those of once-a-month intervals ($p<0.05$ for bootstrapping and permutation). The Shannon index was higher for twice-a-month interval but the confidence interval overlapped among the sampling intervals, except in the case of warmer and twice-a-month datasets for Aculeata (table 1). These patterns diversity statistics were the same for Aculeata and Apidae.

On twice-a-month interval Chao-1 and Jack-1 estimated 67 and 59 aculeate species, and 38 and 36 bee species respectively. Comparing these values with observed richness, the twice-a-month interval accounted for about 60% of the estimated Aculeate species and 65% of the bee species while the once and warmer datasets accounted for about 39% of the estimated Aculeate and bee species. The richness extrapolation of once-a-month interval presented lower values than those of observed richness in twice-a-month interval. Most of values of estimated richness for the once-a-month sets were lower than the observed richness of twice-a-month interval.

The rarefaction and extrapolation curves were still rising until 500 samples (figures 1-2), including the observed number of samples (the smallest reference sample was 34), stabilizing only with the extrapolation of more than 600 samples. For Aculeata the confidence intervals overlap under the observed number of samples but do not after 200 samples when twice-a-month interval extrapolates a higher number of species. For the bees the confidence intervals overlap for all samples. In both cases the confidence interval of twice-a-month interval was greater than those for once-a-month intervals.



Figures 1-2 – Sample based rarefaction and extrapolation for Aculeata and Apidae richness considering the three sampling intervals.

No differences between random and warmer once-a-month datasets were found for any of the diversity statistics analyzed here.

Table 2 – Sampled species by month (June to December 2014 and January to May 2015).

	6	7	7	8	8	9	10	10	11	12	12	1	1	2	2	3	3	4	4	5	5
APIDAE																					
Anthidium manicatum (Linnaeus, 1758)						1															
Anthrenoides meridionalis (Schrottky, 1906)	1		1			1												1			
Augochlora iphigenia Holmberg, 1886								1		1											
Augochlora sp-1							1														
Augochlora sp-2																1					
Augochlora sp-3											1										
Augochlora sp-4				1																	
Augochlora thalia Smith, 1879		1		1				3		1	1										
Augochlorella ephyra (Schrottky, 1910)	2						1	1	1	7	2	3		8	1	7	3				2
Augochlorella urania (Smith, 1853)														1							
Augochloropsis sp-1				2													1				
Augochloropsis sp-2													1								
Ceratina asuncionis Strand, 1910								1						1							
Ceratina sp-1									1												
Ceratina sp-3	1																				
Dialictus sp-1										1	4		1								
Exomalopsis auropilosa Spinola, 1853	3														1			1			
Melissodes nigroaenea (Smith, 1854)														3	1						
Melissoptila fiebrigi Brethes, 1909	2																				
Oxaea flavescens Klug, 1807	1		2																		1
Pereirapis semiaurata (Spinola, 1853)							1			1							2				
Sphecodes sp-1										1											
Thygater analis (Lepeletier, 1841)														1							
Trigona spinipes (Fabricius, 1793)																			1	2	
CRABRONIDAE																					
Liris sp	1									1			1								
Pison delicatum Menke, 1988						1					1										
Pison euryops Menke, 1988	1								1												
Trypoxylon sp									1												
POMPILIDAE																					
Ageniella sp-1																	1				
Ageniella sp-2				1																	
Ageniella sp-3																				1	
Aporinellus sp	1																				
Entypus sp									1												
Poecilopompilus sp			1																		
Priocnemis sp													1	1	1						1
VESPIDAE																					
Hypancistrocerus reflectorius (Dalla Torre, 1904)									1												
Polistes versicolor (Olivier, 1791)				1				1						1							
Polybia chrysothorax (Lichtenstein, 1796)			1															1	2		
Polybia paulista Ihering, 1896					1			1	1		1										

DISCUSSION

The sampling sufficiency, measured by the asymptote of interpolation curve and the proportion observed and estimated species (Chao-1 and Jack-1), was not reached for any interval in spite of the higher values from twice-a-month. The rarefaction and extrapolation curve approach shows difference between the sampling intervals only for the extrapolation of Aculeata richness. We understand that the no difference on observed richness can be attributed to the sampling insufficiency. The twice-a-month proportion of observed and estimated number of species (60%) is lower than the 65% obtained by Banaszak *et al*. (2014) but the percentage of the once-a-month subsets (39%) can be considered very low and therefore non representative of the community. The same overall pattern described here for aculeates was observed for bees, the most common group within the samples.

The general expectation that increasing the sample size results in collection of a greater proportion of the community (WILLIAMS *et al*., 2001; BANASZAK *et al.*, 2014) is confirmed here when we analyzed the observed richness and abundance. Shapiro *et al*. (2014) suggested that the optimal sample number for bee bowl sampling transects is 30 bowls but the effect of sample number has not been tested in the neotropics, then our results can be considered not reliable in terms of sample number alone. But even considering that a high number of samples can retrieve a larger proportion of aculeate community, the sampling interval remains crucial to deal with phenology because the insect communities are strongly affected by seasonal and interannual fluctuations (OERTLI *et al*., 2005).

Banaszak *et al*. (2014) suggested bee sampling in small intervals during the period with higher activity – the flowering season. In the temperate region, with well defined annual seasonality, the flowering season occurs in a restrict period (e.g., WILSON *et al*., 2008; GRUNDEL *et al*., 2011) but in the tropics there is no well defined flowering season. Hand-netting sampling usually follow the entire year (LAROCA & ORTH, 2002) and Cure *et al*. (1991) suggested to collect in the period with highest abundance and richness only to optimize the collection effort. Here the Aculeate assemblage had a tendency to higher richness in Spring and Summer but singletons occurred along the entire year (table 2). About 50% of species sampled during the twice-a-month interval are singletons (19 species), a high number if compared with 16-42% reported by Williams *et al.* (2001) for bees from different localities. Again, this difference could reflect a tropical influence, since the frequency of singletons is anomalously high in most large tropical arthropod surveys (CODDINGTON *et al.*, 2009). Presented results partially reflect the effects of the climate in the region, without well defined seasons when compared to the northern hemisphere.

The studies of Schirmel *et al*. (2010) and Banaszak *et al*. (2014) included the weakly sampling interval in their studies which was considered by both as more effective for bees and we believe the same would result at our study site. However, weekly sampling is extremely time-consuming for studies that have annual duration, considering 52 weeks in a year as weekly sampling more than quadruplicate the monthly sampling effort. Examining the sampling design of Gonçalves *et al*. (2014), we noticed that a total 3,480 bowl trap samples were utilized to compare five fragments with different sizes under once-a-month sampling interval. In this particular case a weekly sampling interval would require more than 15 thousand samples! This great number needs a great field effort and also intense laboratory work with specimen pinning, databasing and identification.

Under our results, the twice-a-month interval was only considered better than monthly sampling intervals when we compare the extrapolation data (estimators and extrapolation curve) for Aculeata. The Shannon diversity index and the estimated species number (rarefaction) were considered the same among the treatments, so we cannot suggest a reliable sampling interval at this point. Therefore, giving this evidence, the twice-a-month interval should be preferable only for the objectives that deal with phenology due to the expectation that a short interval maximizes the chance to sample short time flying wasps. We strongly suggest the need of more investigation about sampling interval effect on Neotropical aculeate assemblages, with larger sample number and on different forest covers.

ACKNOWLEDGEMENTS

The authors thank Instituto Chico Mendes de Conservação da Biodiversidade for license number 12195-1. Special thanks for Gabriel A. R. Melo and Bolivar Garcete-Barrett for Apidae and Vespidae identification and Sam Droege and two anonymous reviewers for the comments on the final manuscript.

REFERENCES

Abrahamczyk, Stefan; Bastian Steudel & Michael Kessler. Sampling Hymenoptera along a precipitation gradient in tropical forests: the effectiveness of different coloured pan traps. Entomologia Experimentalis et Applicata. 2010; 137:262-268.

Banaszak, Jozef; Weronika Banaszak-Cibicka & Piotr Szefer. Guidelines on sampling intensity of bees (Hymenoptera: Apoidea: Apiformes). Journal of Insect Conservation. 2014; 18:651-656.

Calvillo, Laura Meneses; Virginia Melendez Ramırez; Victor Parra-Tabla & Jorge Navarro. Bee diversity in a fragmented landscape of the Mexican neotropic. Journal of Insect Conservation. 2010; 14:323-334.

Cane, James; Robert Minckley & Linda Kervin. Sampling bees (Hymenoptera: Apiformes) for pollinator community studies: pitfalls of pan-trapping. Journal of Kansas Entomological Society. 2001; 73:208-214.

Coddington, Jonathan; Ingi Agnarsson; Jeremy Miller; Matjaz Kuntner & Gustavo Hormiga. Undersampling bias: the null hypothesis for singleton species in tropical arthropod surveys. Journal of Animal Ecology. 2009; 78:573-584.

Colwell, Robert; Anne Chao; Nicholas Gotelli; Shang-Yi Lin; Chang Xuan Mao; Robin Chazdon & John Longino. Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblage. Journal of Plant Ecology. 2012; 5:3-21.

Colwell, Robert & Jonathan Coddington. Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of Royal Society. B. 1994; 345:101-118.

Cure, Jose Ricardo; Guilherme Bastos; Maria José Faccini de Oliveira & Og Francisco de Souza. Influência do tamanho da amostra na estimativa da riqueza em espécies em levantamento de abelhas silvestres (Hymenoptera, Apoidea). Revista Brasileira de Zoologia. 1991; 7:101-110.

Droege, Sam; Vincent Tepedin; Gretchen LeBuhn; William Link; Robert Minckley; Qian Chen & Casey Conrad. Spatial patterns of bee captures in North American bowl trapping surveys. Insect Conservation and Diversity. 2010; 3:15-23.

Gibb, Heloise & Dieter Hochuli. Habitat fragmentation in an urban environment: large and small fragments support different arthropod assemblages. Biological Conservation. 2002; 106:91-100.

Gonçalves, Rodrigo Barbosa; Nicolle Veiga Sydney; Priscila Soares Oliveira & Nathiele Oliveira Artmann. Bee and wasp responses to a fragmented landscape in southern Brazil. Journal of Insect Conservation. 2014; 18:1193-1201.

Gotelli, Nicholas & Robert Colwell. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters. 2001; 4:379-391.

Grundel, Ralph; Krystalynn Frohnapple; Robert Jean & Noel Pavlovic. Effectiveness of bowl trapping and netting for inventory of a bee community. Environmental Entomology. 2011; 40:374-380.

Hammer, Oyvind; David Harper & Paul Ryan. PAST: Paleontological statistics software package for education and data analysis. Palaeontology Electronica. 2001; 4:1-9.

Heneberg, Petr & Petr Bogush. To enrich or not to enrich? Are there any benefits of using multiple colors of pan traps when sampling aculeate Hymenoptera? Journal of Insect Conservation. 2014; 18:1123-1136.

Krug, Cristiane & Isabel Alves-dos-Santos. O uso de diferentes métodos para amostragem da fauna de abelhas (Hymenotera: Apoidea), um estudo em floresta ombrófila mista em Santa Catarina. Neotropical Entomology. 2008; 37:265-278.

Laroca, Sebastião & Afonso Inácio Orth. Melissocoenology: historical perspective, methods of sampling, and recommendations. In: Kevan, Peter & Vera Lucia Imperatriz-Fonseca (eds.). Pollinating bees: the conservation link between agriculture and nature. Proceedings of the Workshop on the Conservation and Sustainable Use of Pollinators in Agriculture, with an Emphasis on Bees. Brasília: Ministério do Meio Ambiente; 2002 p. 217-225.

Leong, Joan & Robin Thorp. Colour-coded sampling: the pan colour preferences of oligolectic and nonoligolectic bees associated with a vernal pool plant. Ecological Entomology. 1999; 24:329-335.

Martins, Aline Cristina; Rodrigo Barbosa Gonçalves & Gabriel Augusto Rodrigues de Melo. Changes in wild bee fauna of a grassland in Brazil reveal negative effects associated with growing urbanization during the last 40 years. Zoologia. 2013; 30:157-176.

Moreira, Eduardo Freitas; Rafaela Lorena da Silva Santos; Uire Lopes Penna; Catalina Angel-Coca; Favızia Freitas Oliveira & Blandina Felipe Viana. Are pan traps colors complementary to sample community of potential pollinator insects? Journal of Insect Conservation. 2016; 20:583-596.

Nielsen, Anders; Ingolf Steffan-Dewenter; Catrin Westphal; Olivia Messinger; Simon Potts; Stuart Roberts; Josef Settele; Hajnalka Szentgyorgyi; Bernard Vaissiere; Michalis Vaitis; Michal Woyciechowski; Ioannis Bazos; Jacobus Biesmeijer; Riccardo Bommarco; William Kunin; Thomas Tscheulin; Ellen Lamborn & Theodora Petanidou. Assessing bee species richness in two Mediterranean communities: importance of habitat type and sampling techniques. Ecological Research. 2011. doi: 10.1007/s11284-011-0852-1.

Oertli, Sabine; Andeas Muller & Silvia Dorn. Ecological and seasonal patterns in the diversity of a species-rich bee assemblage (Hymenoptera: Apoidea: Apiformes). European Journal of Entomology. 2005; 102:53-63.

Schirmel, Jens; Sarah Lenze; Daniel Katzmann & Sascha Buchholz. Capture efficiency of pitfall traps is highly affected by sampling interval. Entomologia Experimentalis et Applicata. 2010; 136:206-210.

Shapiro, Leo; Vincent Tepedino & Robert Minckley. Bowling for bees: optimal sample number for “bee bowl” sampling transects. Journal of Insect Conservation. 2014; 18:1105-1113.

Vrdoljak, Sven & Michael Samways. Optimising coloured pan traps to survey flower visiting insects. Journal of Insect Conservation. 2012;16:345-354.

Westphal, Catrin; Riccardo Bommarco; Gabriel Carré; Ellen Lamborn; Nicolas Morison; Theodora Petanidou; Simon Potts; Stuart Roberts; Hajnalka Szentgyörgyi; Thomas Tscheulin; Bernard Vaissière; Michal Woyciechowski; Jacobus Biesmeijer; William Kunin; Josef Settele & Ingolf Steffan-Dewenter. Measuring bee diversity in different European habitats and biogeographical regions. Ecological Monographs. 2008; 78:654-671.

Williams, Neal; Robert Minckley & Fernando Amaral Silveira. Variation in native bee faunas and its implications for detecting community changes. Conservation Ecology. 2001; 5:7.

Wilson, Joseph; Terry Griswold & Olivia Messinge. Sampling bee communities (Hymenoptera: Apiformes) in a desert landscapes: are pan traps sufficient? Journal of Kansas Entomological Society. 2008; 81:288-300.