Trade-offs in the use of multiple survey methods for spiders and harvestmen assemblages in an Amazonian upland forest

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Running Title: Trade-offs in the use of survey methods for arachnids

Abstract

1. Invertebrates can be sampled using any of several established rapid and cost effective methods for documenting species richness and composition. Despite their many differences, different orders of arachnids have being often sampled together in various studies. Active nocturnal search has been long considered the most efficient method for sampling spiders and harvestmen.
2. We compared the number of species and composition of spiders and harvestmen simultaneously sampled using three sampling methods: beating tray, active nocturnal search and Winkler extractors at areas along the Urucu river, Coari, Amazonas.
3. We found that a reasonable inventorying of harvestmen can be accomplished solely by nocturnal search, whereas beating tray and Winkler approaches are redundant. For spiders, both nocturnal and beating tray were complementary and are needed to provide a more complete picture of spider assemblages. Financial and temporal costs of each method employed can be reduced in 39% and 46% respectively.
4. Based on our findings, we propose that different taxonomic groups (e.g. harvestmen and spiders) should be sampled separately in tropical forests, especially for monitoring purposes. The three methods employed are expensive and time-consuming and should only be used combined when advantageous.

Key words: Biological surveys, sampling methods, ecological assemblage, species richness, tropical spiders and harvestmen, Amazonia.

1. Introduction

Arthropods and other highly diverse groups of organisms are ubiquitous in several ecosystems. Therefore, it can be difficult to generate an accurate species list ("strict inventory") for a particular area or to estimate patterns of species abundances ("community characterization") for comparisons among different assemblages (Longino *et al.*, 2002; Scharff *et al.*, 2003; Barlow *et al.*, 2007; Cabra-García *et al.*, 2012). This task is even more challenging in tropical forests, which account for 17% of the land (Whittaker, 1975) but harbor disproportionately more species than any other terrestrial ecosystem (Gaston, 2000). Therefore, logistic support, financial costs, time and the availability of adequate sampling methods are the main resource limitations for sampling biodiversity in tropical forests (Gardner *et al.*, 2008).

Structured inventories incorporate key features of both strict inventories and assemblage characterizations (Oliver & Beattie, 1996; Longino & Colwell, 1997) and are highly desirable for revealing the general distribution and the relative abundance of species across different scales and sites. Structured inventories aim to generate accurate species lists of given areas, but also providing reliable estimates of species abundance, which is a key factor for assemblage characterizations (Fisher, 1999; Cardoso. 2009; Tourinho *et al.*, 2014). Structured inventories normally use a combination of several sampling methods and have become an interesting alternative approach for monitoring arthropods or using arthropods as indicators of ecological change and ecosystem dynamics (Souza *et al.*, 2012).

The use of several sampling methods is often required to produce a reliable estimation of species richness and composition (e.g., Coddington *et al.*, 1991; Bonaldo *et al.*, 2009),. Although sampling method performance may vary among different taxonomic groups (see Gardner *et al.*, 2008), in Tropical forests many different taxonomic groups have been usually sampled together, using a combination of selected sampling methods (e.g., Bonaldo *et al.*, 2009) and massive sampling effort. Given a sufficiently high sample effort, the combination of several sampling methods should adequately represent both species richness and composition (Azevedo *et al.*, 2014). However, the use of several sampling methods is not necessarily optimal for improving monitoring and evaluation programs (Gardner *et al.*, 2008; Porto *et al.*, 2016). Given the costs, monitoring programs often seek an optimum balance between sampling effort and time consumed to achieve their goals (Souza *et al.*, 2012). Therefore, the reduction of sampling effort was suggested for invertebrate surveys based on the redundancy and complementarity of sampling methods in Amazon basin (Souza *et al.*, 2012; Tourinho *et al.*, 2014; Porto *et al.*, 2016).

We used the opportunity to sample arachnids (mostly spiders and harvestmen) at Porto Urucu petroleum/natural gas production facility to address the following three questions: (1) Do sampling methods and protocols developed for spiders can be reliable for harvestmen? Specifically, we assessed whether the same sampling methods (nocturnal search, litter samples and beating tray) yielded different sets of spiders and harvestmen species and different estimates of local species richness; (2) What is the estimated species richness of spiders and harvestmen at this site? and (3) What is the most effective single sampling technique for estimating two different taxonomic groups (spider and harvestmen assemblages) in this tropical forest? To examine ecological correlates of spider distribution patterns, we also used a guild classification approach (Dias *et al.*, 2010), which provides a useful framework for describing and analyzing the assemblage structure.

2. Material and Methods

3.1. Study site – The present study was conducted in Coari municipality, state of Amazonas, Brazil, at Porto Urucu, a petroleum/natural gas production facility belonging to Petrobras S.A. The petroleum facility is located on the right margin of the Urucu River, Solimões River basin, at 4°30'S, 64°30'W and 650 km west of Manaus (Figure 1). The region is mostly covered by dense upland ("*terra firme*") rain forest with uniform canopy, presenting low diversity of lianas and epiphytes (Lima Filho *et al.,* 2001). About 913 plant species have been recorded and notable changes in the vegetation structure occur in areas with poor soil drainage or in natural or artificial forest gaps (opened for natural gas and oil prospecting and exploiting). Natural gaps are formations produced in the forest matrix from fallen trees or large branches from the canopy, exposing the forest ground to direct solar radiation; whereas artificial gaps were created by the removal of trees and soil materials for the construction or maintenance of the Porto Urucu road network. For this study, records were made only in artificial gaps.

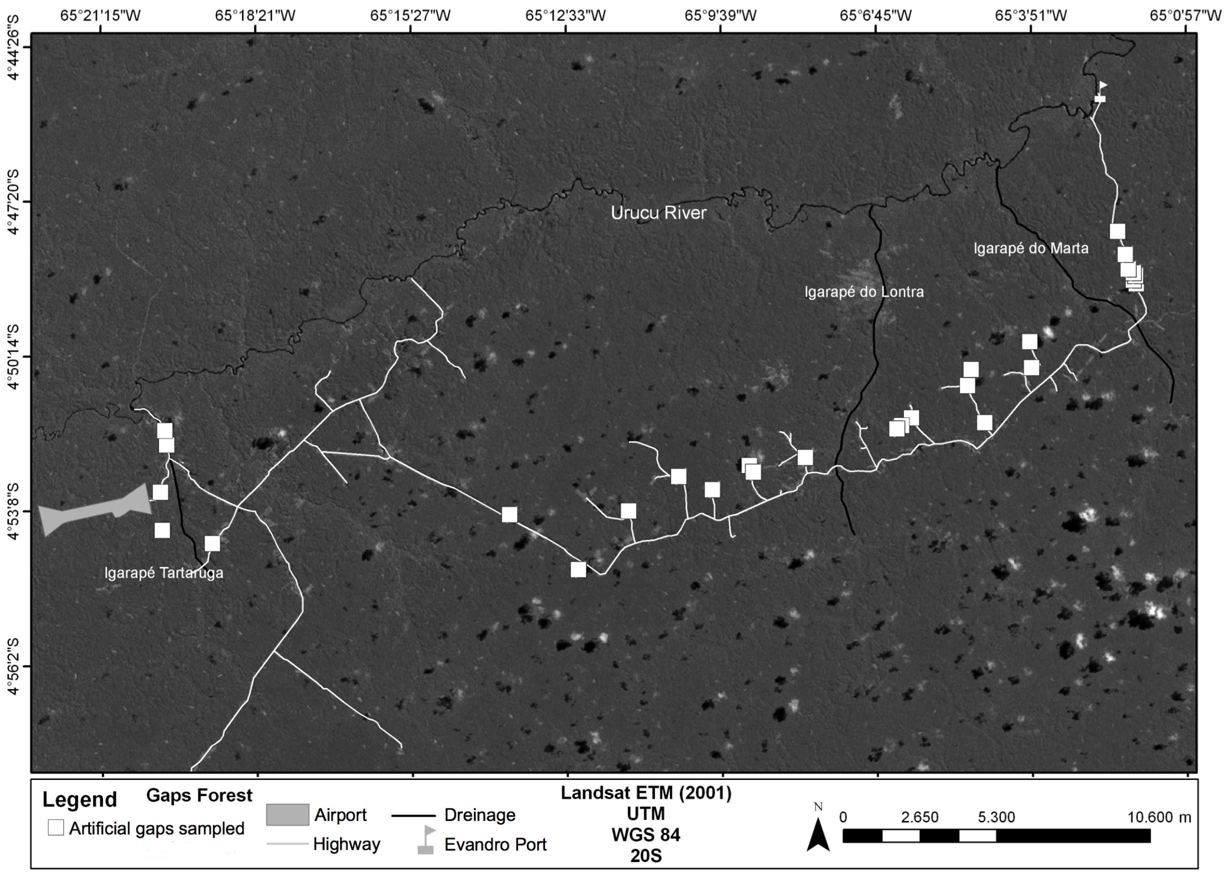

Figure 1. Map of the Porto Urucu sampling site. The squares represent the 33 plots of 300 m^2 sampled.

3.2. Data collection and species identification

We sampled 33 artificial forest gaps between July and November of 2006. Three collectors sampled the arachnids (see Dias & Bonaldo (2012) Table 4 for more details of sampling). Ten 1 m^2 of litter were sampled by Winkler extractors at each one of the collecting sites. The sifted litter were placed in Winkler extractors for 48 hours. Two beating trays and two nocturnal searches were made by each of the three collectors in all 33 sites. Both methods were standardized by time (1 h), within an area of 300 m^2 to control sampling effort (see Davies, 1986; Coddington *et al.,* 1991; Pinto-da-Rocha & Bonaldo, 2006 for methods definition and description). Voucher specimens of both orders are deposited in the arachnological collection of the Museu Paraense Emílio Goeldi - MPEG (A.B. Bonaldo, curator), Belém, Pará, Brazil.

Each individual sampled were carefully examined by the experts in taxonomy and systematics of spiders and harvestmen (Ana Lúcia Tourinho, Ricardo Pinto-da-Rocha, Alexandre Bonaldo and staff, Antonio Brescovit and staff, Erika Buckup and staff). They were dissected and studied on both stereomicroscope and light microscope, we used the somatic characters and the sexual characters to identify our material. Whenever possible identifications to species level were provided; otherwise morphospecies were defined. Only adult individuals were used because most juveniles cannot be adequately identified since their sexual characters are not fully developed, extremely important for accurate identification of both harvestmen and spiders. The taxa in poor taxonomic state of knowledge were not included in any specific genera to avoid misinterpretation (e.g. Cosmetidae sp.1 and Cosmetidae sp.2), for this set of species it is only possible to confirm whether the species belong to the same genus or not after a taxonomic revision. All the potential new genera and species, very common in any inventory undertaken in the Amazon, were referred to as genus or species followed by their number (e.g. Gagrellinae, genus 1 sp.1).

3.3. Data analyses - For both spiders and harvestmen, we estimated the species richness of the site based on data from each sampling method equally sampled among the 33 plots. We also quantitatively compared the relative sampling efficiencies of the nocturnal search, litter sample and beating tray approaches, and the assemblage species compositions between sampling methods for each arachnid order separately. The raw data is available in the supplementary material (Table 1, 2 and 3).

We used the Chao1 index to estimate the species richness of the 33 plots for both spiders and harvestmen (Chao, 1984). The 95% confidence intervals (CIs) associated with these estimates of species richness were also calculated (Colwell, 2012). For this analysis, we pooled the data for all the replicate traps regularly distributed among the 33 sampling plots within each collection type. Chao1 richness estimator provides a conservative minimum estimate of the number of species that are present, accounting for the non-collected species in the samples (Colwell & Coddington, 1994).

Comparative analyses of sampling method performance can be biased by variation in sampling intensity between techniques. Even with standardized sampling, biodiversity measures remain sensitive to the number of individuals and the number of samples collected (Gotelli & Colwell, 2001). Rarefaction methods calculate the expected number of species based on a random subsample of the data, making the comparisons among sampling methods more reliable. However, the sampling methods differed greatly in the number of individuals they accumulated. Therefore, we used sample-based and incidence-based (the average species accumulation curves based on the abundance of individuals) rarefactions to compare the number of species between sampling methods (Gotelli & Colwell, 2001). We used analytical methods to generate valid CIs for the rarefaction curves, which do not converge to zero at the maximum sample size (Colwell *et al.*, 2004). Calculations and simulations were performed using EstimateS (version 8.2) (Colwell, 2012). We also estimated whether the species accumulation curve reached the asymptote, by calculating the first derivative at the last sample using the package BAT in the R environment (Cardoso *et al.*, 2015).

To evaluate the composition similarity between sampling methods, we calculated the

Jaccard similarity index between each pair of collection methods, accounting for the bias toward small values, as this index does not take into account (rare) shared species that were not represented in either of the two sample collections (Chao *et al.*, 2005). The modified Jaccard index is not upper bounded, and we used 1,000 random bootstrap samples to calculate 95% CIs for this index. When the CIs encompass 1.0, we can accept the null hypothesis that the two sampling methods share a similar group of species. Calculation of the adjusted Jaccard similarity and construction of the bootstrapped CIs were done using EstimateS (version 8.2) (Colwell, 2012).

To optimize the number of samples per method, we used the framework proposed by Cardoso (2009). This analyze permute the data matrix focusing in combination of sampling methods that maximize the total number of species. We showed the better combination of methods and number of samples per method to achieve 50%, 80% and 100% of species samples at Urucu. These analyses were calculated using BAT package based on 1000 permutations (Cardoso *et al.*, 2015). To present the composition and identity of species sampled by each method we created simple ordination graphs. Given the high number of spider species, we showed only the ordination figures for spider families.

Spiders are incredibly diverse, therefore, simplification and grouping is an interesting approach to exploring the taxa distribution patterns among the sampling techniques. Species were placed into guilds based on the recent classification for Neotropical spiders (Dias *et al.*, 2010). This guild classification is based on natural history information obtained through direct observation of individuals hunting, resting, building webs, carrying egg-sacs, running, stalking and ambushing (Dias *et al.*, 2010). An inferential test to assess possible differences in guild abundances among sampling methods was made by a non-parametric multivariate analysis of variance (npMANOVA, Anderson, 2001). For this analysis we did not used the Jaccard similarity index accounting for the bias toward small values, as the sampling representation at guild level was reasonably high. The npMANOVA was based on the Bray-Curtis distance measurement. This analysis was performed in

R (version 2.14) (R Development Core Team, 2011) using the "vegan" package (Oksanen, 2012).

3.4. Project Costs - To evaluate the project costs, we considered time spent collecting in the field, during sorting and identifying in the lab. The summed time of all sampling techniques together was set up as maximum effort (100%) and we calculated the fractions of maximum effort for the three methods employed.

3. Results

We sampled 2,139 harvestmen and 3,786 spiders distributed into 26 harvestmen species and 625 spider species, respectively. The beating-tray method sampled 1,236 harvestmen (7 families, 13 species) and 1,969 spiders (28 families, 417 species). The nocturnal search method sampled 667 harvestmen (9 families, 24 species) and 1,537 spiders (30 families, 357 species). The Winkler extractors sampled 236 harvestmen (9 families, 13 species) and 280 spiders (15 families, 91 species). Given the different sampling methods used, the species sampled included arboreal, soil and litter specialist species (supplementary material).

For harvestmen, nocturnal search sampled significantly more species than either Winkler or beating tray (Figure 2). Nocturnal search also was more effective, sampling more species and less individuals (Figure 3). Relatively fewer harvestmen individuals were sampled using the Winkler method (Figure 3). However, Winkler seems to be more efficient than beating tray, as the number of species sampled by each method is similar given a similar number of samples (Figure 2). The sampling effort was sufficient to sample a reasonable proportion of the harvestman richness at the Urucu site. Beating tray, nocturnal search and Winkler sampled 87%, 91% and 43% of the total number of species expected to be sampled with each method based on Chao1. Despite the lower proportion sampled with Winkler, the slopes at the end of the accumulation curves for all methods (beating, nocturnal search and Winkler) were close to zero (< 0.009 in all cases).

For spiders, the Winkler method sampled much less species than either beating tray or nocturnal search (Figures 2 and 3). In absolute terms, the beating tray method collected more species than the nocturnal search (Figure 2). Nevertheless, the rarefaction curves of the two methods are not significantly different at the point at which the comparison between these two methods is possible based on the number of individuals sampled (Figure 3). Different from harvestmen, none of the spider accumulation curves were close to being saturated (Figures 2 and 3). The slopes at the end of the accumulation curves for all methods (beating, nocturnal search and Winkler) was ~0.1 in all cases, meaning that if a new sample was carried out we expect to find ~3 new species using beating tray, ~2.5 species during nocturnal search and ~0.6 species in a new Winkler sample. Beating tray and nocturnal search sampled ~60% and Winkler sampled ~43% of the total number of spiders species expected to be sampled by each method based on Chao1.

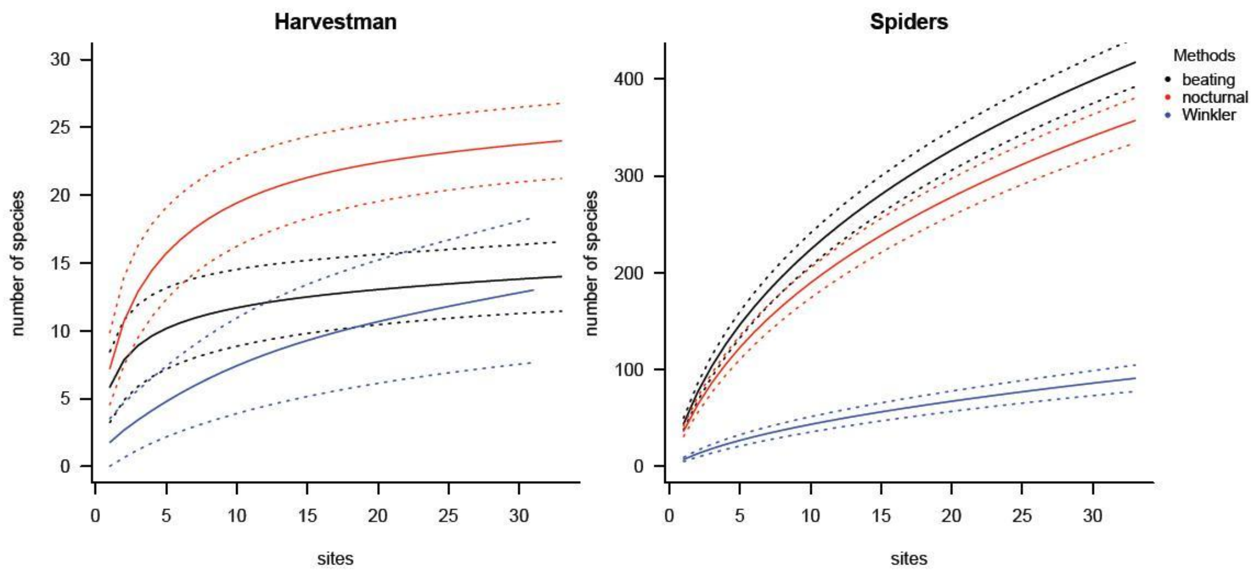

Figure 2. Sampled based rarefaction curves for harvestmen and spiders. Fitted dotted lines indicate 95% confidence intervals; tracing dotted lines indicate number of species estimated.

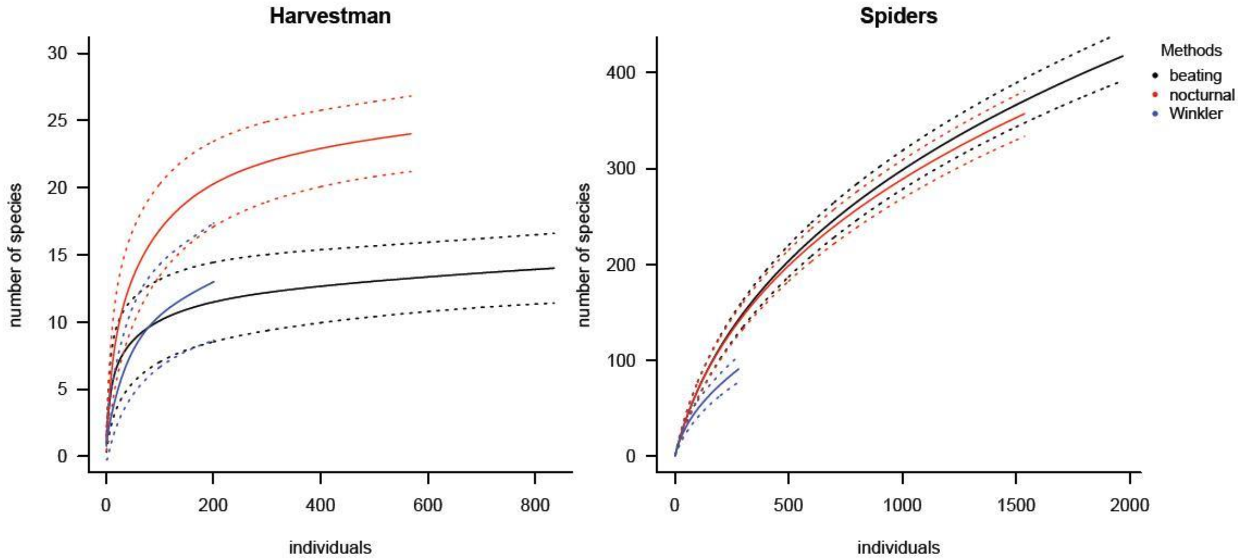

Figure 3. Sample based rarefaction curves for harvestmen and spiders. Fitted dotted lines indicate 95% confidence intervals; tracing dotted lines indicate number of species estimated.

The three techniques generally sampled different sets of species for both harvestmen and spiders (Figure 3). The only exception was for the harvestman assemblage sampled with nocturnal search and beating tray. In this pairwise comparison, the adjusted compositional similarity was around 85% with the 95% CI including 1 (Figure 3). The spider assemblage compositions sampled by nocturnal search and beating tray were also more similar, although the 95% CI was less variable. The other pairwise comparisons for both harvestmen and spiders assemblages composition have many fewer species in common.

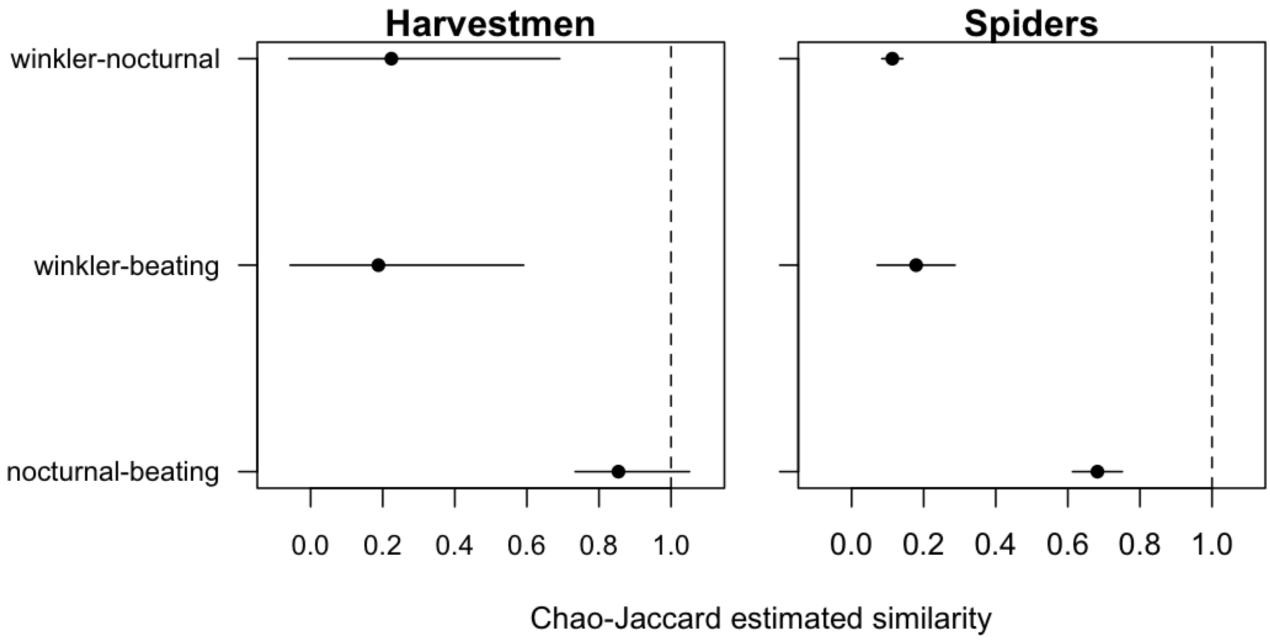

Figure 4. Chao-Jaccard similarity in species composition among the three sampling methods for harvestmen and spiders, adjusted for unsampled species. The point represents the mean similarity and the line around the point the 95% confidence intervals.

At site scale, the harvestman species compositions sampled by nocturnal search, beating tray and Winkler were redundant (Figure 4), but the nocturnal search sampled more exclusively harvestman species than any other method. While nocturnal search collected 7 exclusive species, Winkler sampled only one exclusive species and all species sampled with beating tray were also sampled by the other methods. This result is also expressed in the optimization of sampling methods, while a combination of 11 or 12 nocturnal search coupled with one beating tray and one Winkler sample may account for 80% of harvestman species sampled (Table 1).

The spider assemblage showed a markedly different picture. Each sampling method collected a set of exclusive species (Figure 5). Winkler samples seems to be more redundant in the optimization of sampling when a smaller sampling effort is necessary, but this is because Winkler samples accumulates species at lower rates compared with other methods (less efficient). To detect 80% of spiders species (498 species), all beating samples, almost half of nocturnal search, and two

Winkler samples is necessary (Table 1). Figure 5 show the spiders species sampled by each method collected by family, given that the higher number of species sampled make a species level plot impracticable.

Winkler apparatus consumed nearly half of the financial costs and took most of the time in the field (table 2). This method consumed 55.46 % of time for the study dedicated for the survey in the field. Nocturnal search is the cheapest and faster method, only 25% of financial resources was consumed and 31% of the total time dedicated for the study. Beating consumed only 29% of the financial resources, however it was as faster as nocturnal in the field, but consumed 42.87 % of time in the lab sorting and identifying the material surveyed.

Table 1 - Optimal combinations of samples per method for each site given 50%, 80% or 100% of species sampled at Porto Urucu petroleum facility, Amazonas, Brazil.

	Harvestman			Spiders		
method	50%	80%	100%	50%	80%	100%
Beating	1	1	25	12-13	33	33
Nocturnal	2-3	11-13	33	5	11-12	33
Winkler	0	1	5	0	2	33

The distribution pattern of spider guilds among sampling methods was similar to the distribution of species or families (Figure 6). Each sampling method favored specific set of spider guild compositions (npMANOVA, $r^2 = 0.52$, $F = 53.235$, $p < 0.001$).

Table 2. Financial and time costs spent in the field and lab for each method.

Methods	Financial (%)	field (%)	lab (%)	total (%)
All	100	100	100	100
Nocturnal Search	25	22.27	35.71	31
Beating tray	29	22.27	42.87	35.8
Winkler apparatus	46	55.46	21.42	33.2

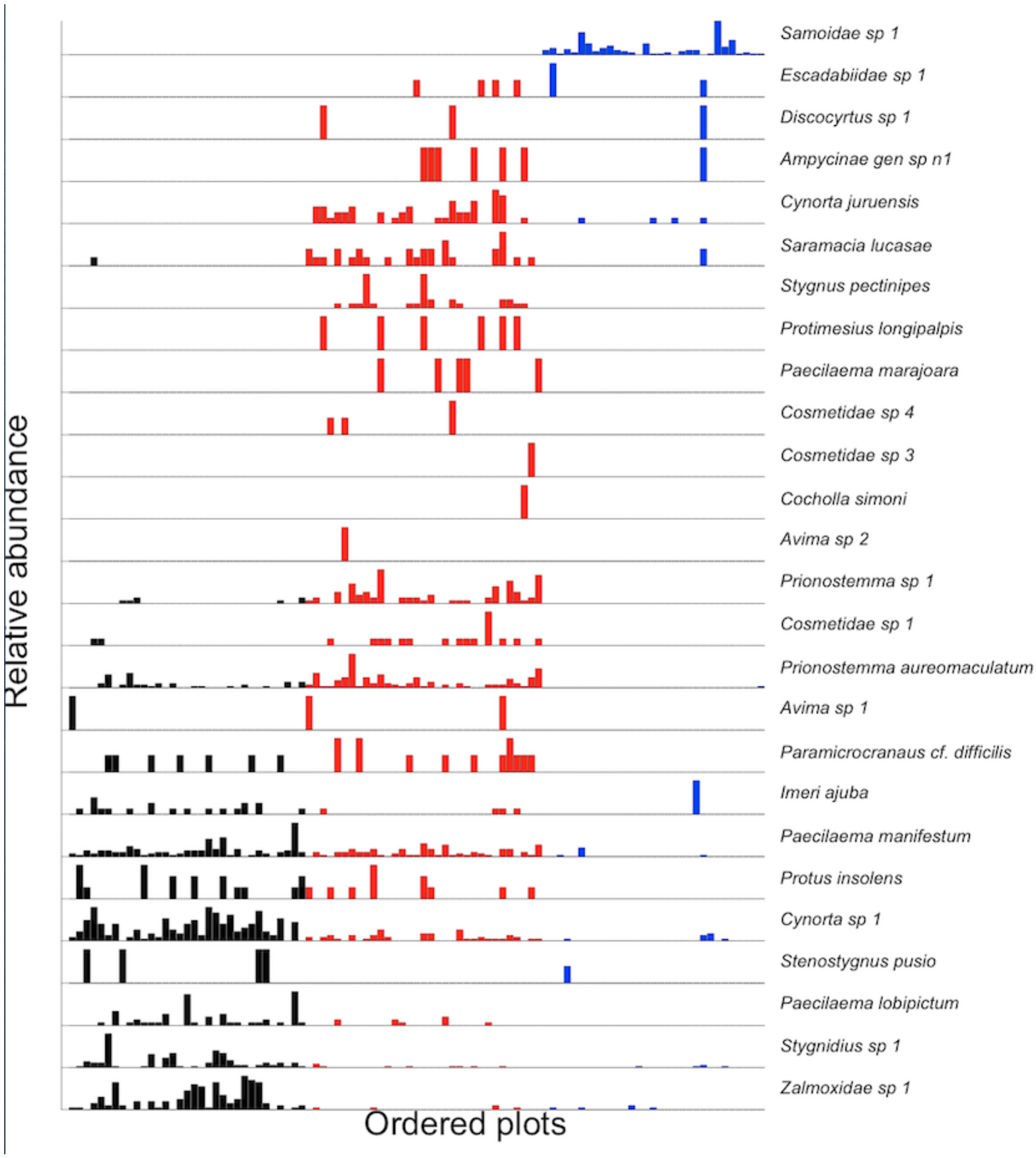

Figure 5 - Distribution of harvestman species sampled by each technique among the study plots at Porto Urucu, Amazonas, Brazil. The colors scheme is the same as in Figure 2 (black bars represent the species sampled with beating tray, red bars the species sampled during nocturnal search and blue bars the species sampled with Winkler extractors). Each column represents one sampling plot. The bar size represents the relative abundance standardized by the maximum number of individuals recorded in one plot.

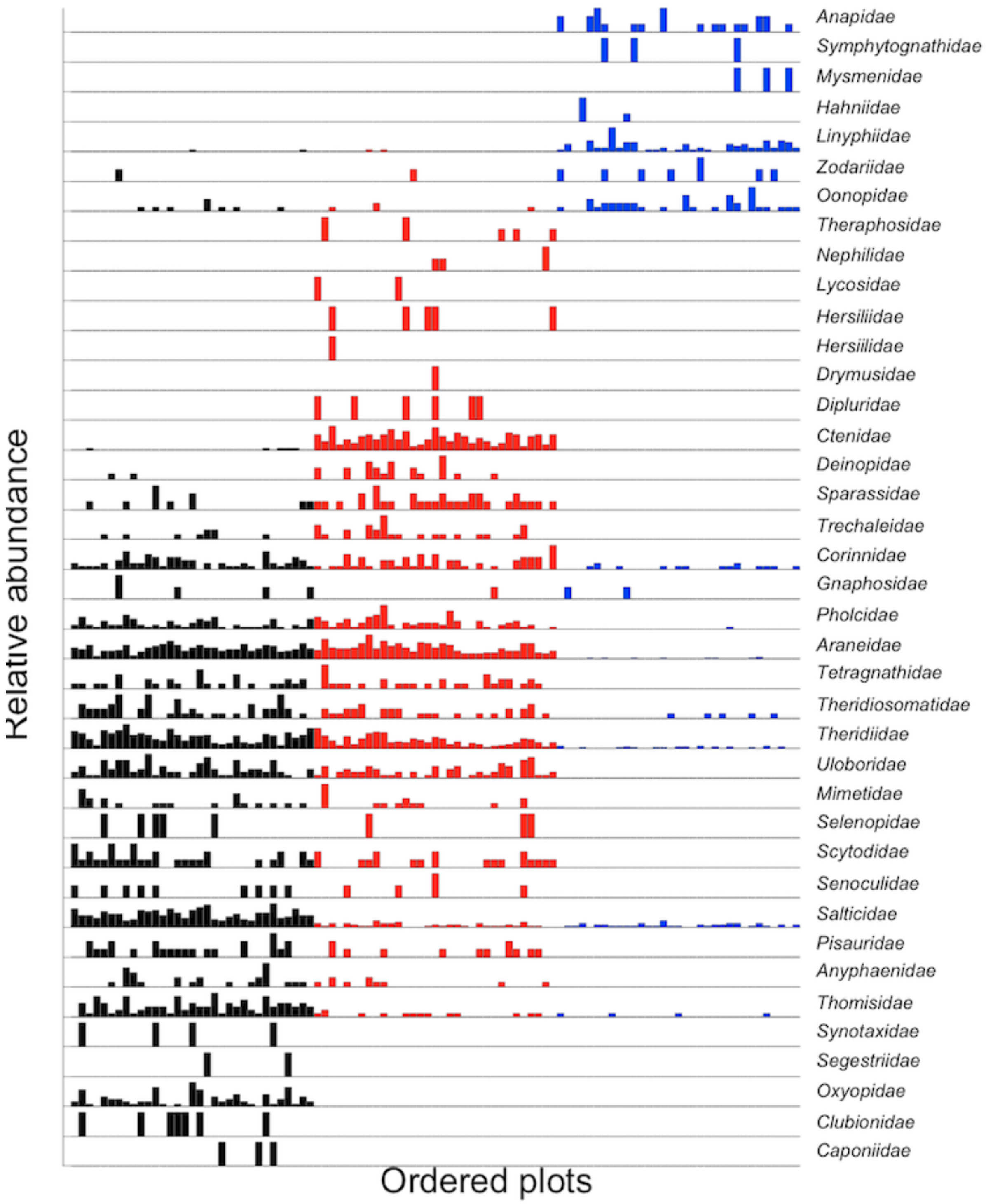

Figure 6 - Distribution of spiders families sampled by each technique among the study plots at Porto Urucu, Amazonas, Brazil. The color scheme is the same as in Figure 5. Each column represent one sampling plot, and the bar size represents the relative abundance standardized by the maximum number of individuals recorded in one plot.

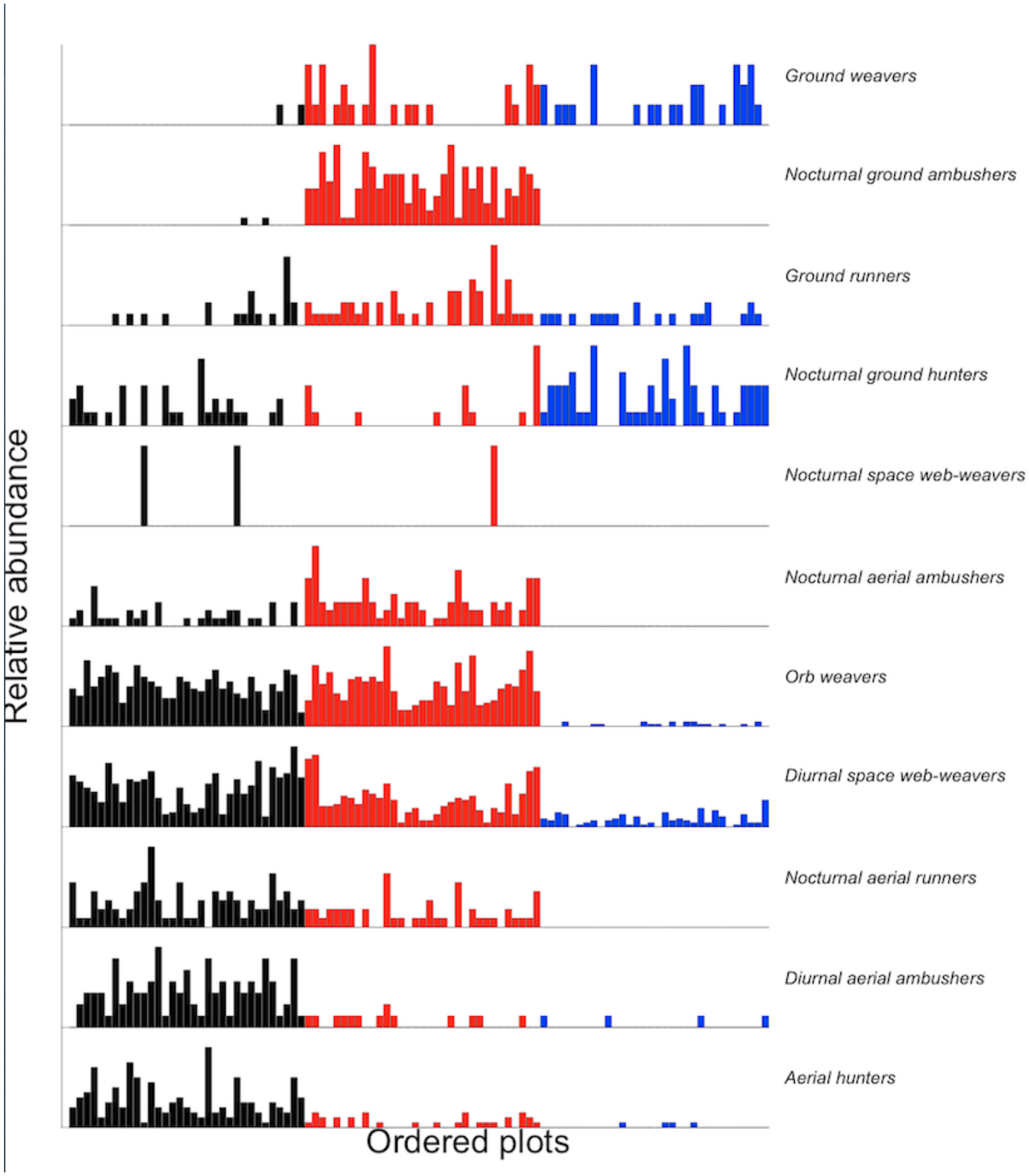

Figure 7 - Distribution of spider guilds sampled by each technique among the study plots at Porto Urucu, Amazonas, Brazil. The color scheme is the same as in Figure 5. Each column represent one sampling plot, and the bar size represents the relative abundance standardized by the maximum number of individuals recorded in one plot.

4. Discussion

The efficiency of methods used to simultaneously sample different taxonomic groups of arachnids were not explored or compared before. In our study the most striking difference between spider and harvestmen inventory data is that any sampling method got close to the asymptote of accumulation curves for spiders, however the same sampling effort was sufficient to describe the richness of harvestmen at the Urucu site. Therefore, a reasonable inventory of harvestmen is then accomplished with less effort and methods than it would be needed for recovering similar results with spiders. Spider abundances are high locally, the higher richness and abundance reflects on diversity estimates. Based on Chao1 estimates 89% of harvestman species were sampled, while only 66% of spider species were detected with all methods combined. The higher richness, abundance, complexity and diversity of spider guild account for the need for several sampling methods and several replicates that are not necessary for a reasonable inventory of harvestmen in tropical forests.

The nocturnal search method was the most efficient method for accessing the species richness of harvestmen in the study area, while Winkler and beating were less efficient and were redundant with nocturnal search method. These results are corroborated by two previous works comparing the efficiency of sampling methods for this group in three different areas in the Amazon basin (Tourinho *et al.*, 2014; Porto *et al.*, 2016). Differently, for spiders, the beating tray method collected more species and more individuals than either the nocturnal search or Winkler methods. In that regard, our findings differ from other studies (Coddington *et al.*, 1991; Cardoso, 2008; Azevedo *et al.*, 2014) and cast doubt on the claim that nocturnal search is the most productive method for accessing spider richness and should be used alone in tropical forests (Azevedo *et al.*, 2014). Based on our results, the performances of the beating tray and nocturnal search approaches are equivalent for describing the local richness of spiders in the Urucu basin and should each be used in their most appropriate context.

While for harvestman the species composition for the area using the maximum effort (three methods combined) can be virtually summarized solely by nocturnal search, for spiders each sampling method sampled a different set of species. Both the nocturnal search and beating tray approaches sampled almost the same number of species of spiders, however, each method recorded a different set of species. Similar richness and different species composition between nocturnal search and beating tray suggests that these two methods have very little redundancy in this tropical forest and richness estimates based in only one of these sampling methods will result in very biased results. Despite the differences in species composition and abundance found at plot level, the same pattern can be detected at site scale. For harvestman, the redundancy of sampling methods is very high, with only one species exclusively sampled by Winkler or beating tray, while the significant part of the species was sampled by nocturnal search alone. These results suggest that complementarity among sampling methods can be detected in small-scale inventories for spiders, while redundancy between sampling methods is strong for harvestman inventories.

The pattern of spider guilds composition among sampling methods was somehow expected for a typical Neotropical forest. Winkler extractors recorded mainly species with small body lengths that live on the ground or in the leaf litter, such as Ground weavers, Ground hunters and Nocturnal Ground hunters, which are among the spiders that are normally accessed by this technique (e.g., Anapidae, Hahniidae, Oonopidae, Tetrablemmidae, Zodariidae and Zoridae). Also observed in this habitat are the large ground spiders such as Ctenidae and several Mygalomorphae that are too big to pass through the sieve’s grid. These spiders are much more likely to be sampled by nocturnal search. The higher spider abundance recorded during the nocturnal search was due to the inclusion of nocturnal ground ambushers, nocturnal aerial ambushers, orb weavers and diurnal space web weaver species. Despite being diurnal, webs produced by these spiders remain intact or in pieces during the night. Given that spiders are often found in retreats near the webs and since the webs are very conspicuous at night during light focal search, this guild can be well documented with nocturnal sampling. Beating was usually sampled in the morning, and the presence of nocturnal species can be explained by the fall of spiders that were resting or hidden in the bushes.

Remarkably, harvestmen species possess low dispersal capability and are generally highly endemic (Pinto-da-Rocha *et al.,* 2007), while spider's species tend to disperse to greater distances, even dispersing across continents through ballooning (Foelix, 2010). Despite the fact that several spider species also have low dispersal capability, notably some clades are typically encountered in leaf litter (e.g., Oonopidae), and thus might be expected to present micro-distributed patterns even in the Amazonia. The differences in species dispersal abilities between harvestman and spiders often result from different species distributions in space. Therefore, the methods and protocols developed for spiders are not always similarly successful for harvestmen.

Our results were different in terms of efficiency and congruence for each of the groups sampled. The financial and temporal investment for collecting and sorting spiders is more than twice the investment needed for inventorying harvestmen. Beating tray is the second most expensive method, it consumed the same time as nocturnal search in the field and 42% of the time processing the material in the lab. However, as there is a high complementarity between spider species sampled with nocturnal search and beating tray, these methods should be used together if a more detailed picture of spider diversity is needed. To get nearly 80% of spider species sampled all beating trays and about half of nocturnal search need to be employed. Multiple methods and several replicates are used to sample spiders in many studies and the accumulation curves are also still not asymptotic (Coddington *et al.*, 1991; Bonaldo *et al.,* 2009; Cabra-García *et al.*, 2010; Azevedo *et al.*, 2014). Therefore, a traditional spider sampling protocol is usually not the most efficient strategy for studies of assemblage associations with environmental variables and ecological impact. It is nearly impossible to assess a complete spider species inventory in the tropics, so larger protocols demand higher financial costs, increasing time spent in both the field collecting and in the lab processing. Our study suggests the need to delve deeper into the issue efficiency of sampling methods for mega diverse arachnid orders in future ecological studies. We suggest protocols for monitoring studies should focus in spider guilds. For the Urucu forest, for example, an efficient way to monitor a large amount of spider's species would be using only beating tray, but Ground weavers and Nocturnal ground ambushes will be underestimated.

Conversely, beating and Winkler apparatus are redundant, temporal and financially costly for harvestman assemblages, and nocturnal sampling provide a reasonable view of harvestman assemblage at lower cost. In addition, with about half of the sampling effort employed in this study, around 80% of harvestman species would be sampled using a reduced version of nocturnal search (cryptic nocturnal search). Cryptic nocturnal search successfully capture the ecological patterns that would be achieved with larger comprehensive surveys for harvestmen at lower costs (Tourinho *et al.*, 2014 and Porto *et al.*, 2016). The cryptic nocturnal search may reduce the time spent by nearly 87% (Porto *et al.*, 2016) optimizing the nocturnal search and seems to be another promising alternative for harvestman monitoring purposes.

It is clear that a protocol developed for spiders surveys is inadequate for harvestmen in terms of efficiency, congruence and financially, especially for monitoring or ecological impact studies. We propose that different taxonomic groups such as harvestmen and spiders should not be surveyed simultaneously in Tropical forests. Nocturnal search is still the most efficient method to sample harvestmen at lower cost and optimum results. We still need future studies to test and elaborate an optimum protocol for spiders in the Amazon tropical forests. In this paper however, we present an economic and efficient alternative protocol, composed of active nocturnal search and beating tray, which saves almost 50% of the costs.

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