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***Ad-Hoc* vs. Standardized and Optimized Arthropod Diversity Sampling**

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Abstract: The use of standardized and optimized protocols has been recently advocated for different arthropod taxa instead of *ad-hoc* sampling or sampling with protocols defined on a case-by-case basis. We present a comparison of both sampling approaches applied for spiders in a natural area of Portugal. Tests were made to their efficiency, over-collection of common species, singletons proportions, species abundance distributions, average specimen size, average taxonomic distinctness and behavior of richness estimators. The standardized protocol revealed three main advantages: (1) higher efficiency; (2) more reliable estimations of true richness; and (3) meaningful comparisons between undersampled areas.

Keywords: abundance classes; accumulation curves; Araneae; biodiversity assessment; Iberian Peninsula; Portugal; rank-abundance curves; species richness estimators

1. Introduction

Ad-hoc sampling of species diversity, either unplanned or planned on a case by case basis, is often used as an approach for obtaining the maximum information about areas in a minimum amount of time. Depending on the purpose, this can even be the only option available, as the resources required to test the best method or combination of methods may not be available. This approach can be very efficient for some well known taxa if the objective is solely to compile species lists of the study areas [1-3]. If the samplers are taxon specialists, a very fast accumulation of species is possible. If the species lists are complete or close to it, it is even possible to compare communities sampled with *ad-hoc*, not standardized, sampling. However, for mega-diverse groups like spiders or other arthropods this is rarely the case.

More than 40,000 species of spiders are known [4] and probably even more remain undescribed. Several hundred species can coexist at certain times of the year in very small and restricted areas [5]. This high local diversity is possible in many biomes, from temperate Mediterranean forests [6] to tropical forests [5,7]. Therefore, to know what species live where is a challenge. With many small, cryptic and even locally rare species, it is unrealistic to compile complete species lists, even if this list is intended for areas as little as one hectare and for a time-frame as short as one or two weeks [6-10]. Without sound species lists and/or a measure of effort, comparisons are usually impossible, incomplete or even biased.

Standardized sampling is most often employed in ecological studies of arthropods. It allows the immediate comparison of communities even if species lists are incomplete [11-14], unless the completeness is so low that only an almost random fraction of the communities is in fact sampled. Besides, standardized sampling allows for obtaining relative abundance data in addition to species richness. This way, statistical inference is possible, not only in ecology but also in conservation biology, biogeography or other areas. Usually, a combination of methods is required in such sampling [15-18]. However, this approach is frequently less efficient than *ad-hoc* sampling in obtaining species lists [2] because it is usual that most of the individuals will belong to the fraction of more common species [10]. For this reason, recent advances in sampling strategies have led to the development of field protocols that are both standardized and optimized [19].

For Iberian and Mediterranean spiders, a field protocol nicknamed COBRA—Conservation Oriented Biodiversity Rapid Assessment—has been recently developed [19]. Based on extremely intensive sampling of different habitats in Portugal [6,8,9], it is both standardized and optimized. It is also flexible, in the sense that different sub-protocols with varying degrees of effort have been proposed to cope with different objectives or available resources (human, time or financial). The work now presented intends to compare the *ad-hoc* and COBRA sampling approaches when applied to a protected area of central Portugal. Our objective was not to compare areas, but rather different approaches that shared the common goal of inventorying the spiders of a heterogeneous natural area with different habitats. We wanted to compare an *ad-hoc* sampling (not limited in area, time or methods) to a fully standardized and optimized protocol (with standardization and optimization in area, time and methods). For the purpose we compared eight parameters that were thought to possibly provide different outcomes according to the approach adopted:

(1) Efficiency of increasing observed species richness by unit of time; as experts usually know how to look for specific taxa and how to capture them with minimum time spent in the task, we hypothesized that the *ad-hoc* sampling, as long as done by experts, would be more efficient.

(2) Efficiency of increasing observed species richness by individual; as quantitative sampling demands that even the most commonly collected species keep being collected, we hypothesized that the *ad-hoc* sampling would also be a more efficient approach when comparisons were made by individuals captured.

(3) Over-collecting of common species; as *ad-hoc* samplers tend not to collect species already sampled and positively identified, we hypothesized that over-collecting would be much stronger with the standardized protocol.

(4) Proportion of singletons; as *ad-hoc* samplers tend to look for new individuals of species for which only one specimen was collected, this proportion was hypothesized to be smaller for *ad-hoc* sampling.

(5) Species abundance distribution; related with the two former parameters, we hypothesized that the *ad-hoc* sampling would present a much more even species abundance distribution.

(6) Average size of specimens; because *ad-hoc* sampling heavily relies on spotting many conspicuous species, we hypothesized that the average size of specimens captured by it would be larger than the one of standardized sampling.

(7) Taxonomic distinctness; as many experts are specialized in some taxa (families or genera), we hypothesized that there would be a higher taxonomic bias in *ad-hoc* sampling, with respective smaller average taxonomic differentiation between observed species.

(8) Behavior of richness estimators; because the relative abundance data of species should be more reliable with standardized sampling, we hypothesized that non-parametric species richness estimators would perform better, with more asymptotic curves, with this protocol.

2. Results and Discussion

A total of 202 species were collected in the Paúl de Arzila Nature Reserve, 163 of these with *ad-hoc* sampling and 140 with standardized sampling [20]. As for the latter, in the first of the two habitats sampled, a riparian corridor, 82 species were collected, while in the second habitat, a meadow, 94 species were collected. The number of individuals was higher for the COBRA protocol despite the lower number of species, causing a much higher sampling intensity (Table 1).

For this work we did not try to limit the *ad-hoc* sampling to the delimited areas sampled by the standardized protocol. Neither did we try to limit the analyses of the first to the short sampling period of the latter. Or to use the same sampling methods in both approaches. We recognize that all these issues—sampling area, phenology and methodology—do influence, even decisively, the outcome of any sampling. However, this seemingly unbalanced comparison was the key issue of this study. We wanted to compare a completely *ad-hoc* sampling, with no restrictions in area, time or methods, to a fully standardized and optimized approach, with the objective of inventorying a natural area with different habitat types. The optimization involved choosing a minimum number of areas as dissimilar as possible in vegetation structure, to capture a maximum number of species (in this case a riparian corridor and a meadow). It also involved choosing the best time for collecting in order to maximize

species richness [21]. Finally, it involved choosing the best possible combination of methods [19]. If we had limited the *ad-hoc* dataset to any of these restrictions it would be a standardized (although not optimized) sampling and not *ad-hoc*.

Table 1. Overall results for the different sampling approaches and sites.

	<i>Ad-hoc</i>	COBRA		
		Total	Riparian corridor	Meadow
Person-days	24	8	4	4
Individuals (n)	1375	2538	1566	972
Species richness (S)	163	140	82	94
Sampling intensity (n/S)	8	18	19	10
Singletons (%)	36 (22%)	37 (26%)	25 (30%)	30 (32%)
Doubletons (%)	22 (13%)	7 (5%)	8 (10%)	8 (9%)
Estimated richness				
Chao 1	192	238	121	150
Chao 2	207	194	112	128
Jackknife 1	221	178	108	124
Jackknife 2	240	202	122	140

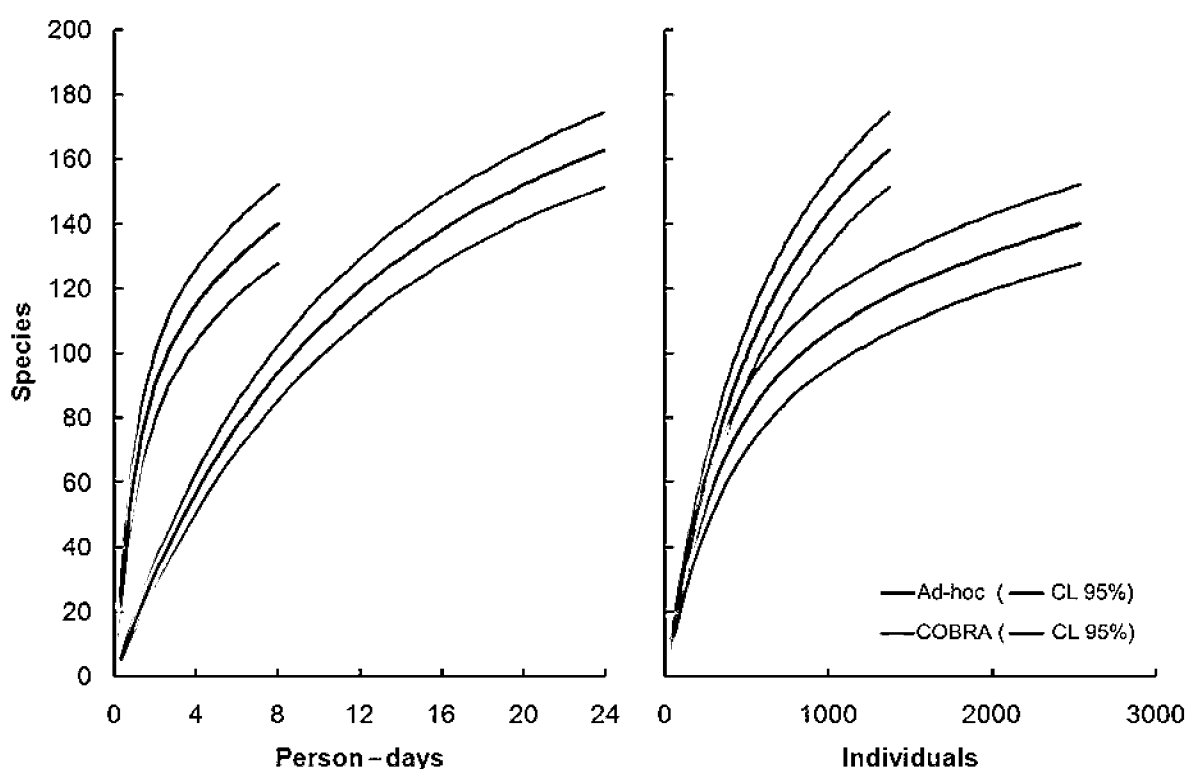
2.1. Efficiency by Unit of Time

Contrary to our predictions, the *ad-hoc* sampling revealed to be the least efficient methodology regarding the number of species obtained by time, in this case person-days (Figure 1). The COBRA curve is much steeper, especially at the beginning, than the *ad-hoc* curve. Following a standardized and optimized approach may be most efficient for sampling a large number of species with a relatively low effort. The optimization of area, time and methods avoids that collectors waste limited resources in: (1) sites that are much similar to the ones already sampled; (2) seasons of low species diversity (although these seasons do provide a different set of species, see [21] for a demonstration that it is more efficient to concentrate most sampling in the richest season than to spread the effort towards other seasons) and; (3) methods that are either inefficient or that largely overlap with other methods already applied.

It can be argued that an *ad-hoc* approach, if done by experienced collectors, may provide a rapid accumulation of species and in fact this is often the case [2]. An experienced collector is probably capable to know how to sample the highest variety possible in a short time or to immediately understand when a method is not being productive [22]. However, this approach has some pitfalls. Firstly, experienced collectors, often taxonomists, may be biased towards some taxa, families, genera or species. Either these are the taxa that are of most interest to them as taxonomists, or these are taxa that they know to be especially rare, beautiful or any other particular characteristic (but see below that this was not the case in this work, 2.7). Secondly, even experienced collectors may miss part of the community that they are not aware to exist in the site. Especially cryptic or very small species may escape the collector attention if (s) he is not required to follow a strict protocol [23]. Thirdly, experienced collectors are in high demand and low availability, and it is usually necessary to trust part

of the work to less experienced personnel. In such case, an *ad-hoc* approach could be much less efficient. This is not to say that it is desirable to trust exclusively on inexperienced collectors. At least some of the collectors should be experienced so that they are proficient with all collecting methods and can pass on such knowledge to trainees. Additionally, the consistency between different teams performing the same protocol is maximized if some of the collectors have previously done this same type of sampling, establishing common and universal practices.

Figure 1. Randomized accumulation curves and respective confidence limits for the species richness obtained by the *ad-hoc* and COBRA approaches. Curves are drawn either with person-days or individuals as measures of effort.



2.2. Efficiency by Individuals Captured

As hypothesized, the comparison of *ad-hoc* and COBRA sampling with the number of individuals as effort measure concludes that the first is more efficient than the latter (Figure 1). The *ad-hoc* sampling curve is steeper and seems to be further from the asymptote than the COBRA one. It should be noticed however, that the area covered by the first was much larger than the two sampling plots of the latter, which for itself may account for such differences. Additionally, the *ad-hoc* samplers did not collect individuals when they were readily identifiable as a previously collected species. This procedure eliminated an unknown, but certainly large, part of the individuals from the analyses.

Capturing more individuals may be desirable if, for example, some species are hard to tell apart and require an understanding of intraspecific variation for their correct identification [24]. New species for science also usually require a large number of specimens for their detailed description and large numbers are mostly provided by standardized sampling [25,26]. On the other hand, capturing many

individuals of the same species may not be desirable if such species are disproportionately dominating the samples [10] or if some species have a low number of individuals in the site and a large proportion is collected, endangering the population. In any case, almost no intensive semi-quantitative protocols made to date have ever reached the point of richness decreasing during the course of sampling [8].

2.3. Over-Collecting of Common Species

The Simpson measure of evenness was found to be similar for both approaches ($E_{1/D}$ *Ad-hoc* = 0.228; COBRA = 0.241; $p = 0.188$). In fact, although not significantly, the standardized approach produced slightly more even samples. It seems therefore that there is no over-collecting of common species by the standardized protocol, which could lead either to a waste of resources both during sampling and latter during sorting [10], or to the depletion of fauna in the sampling sites [8].

2.4. Singletons Proportion

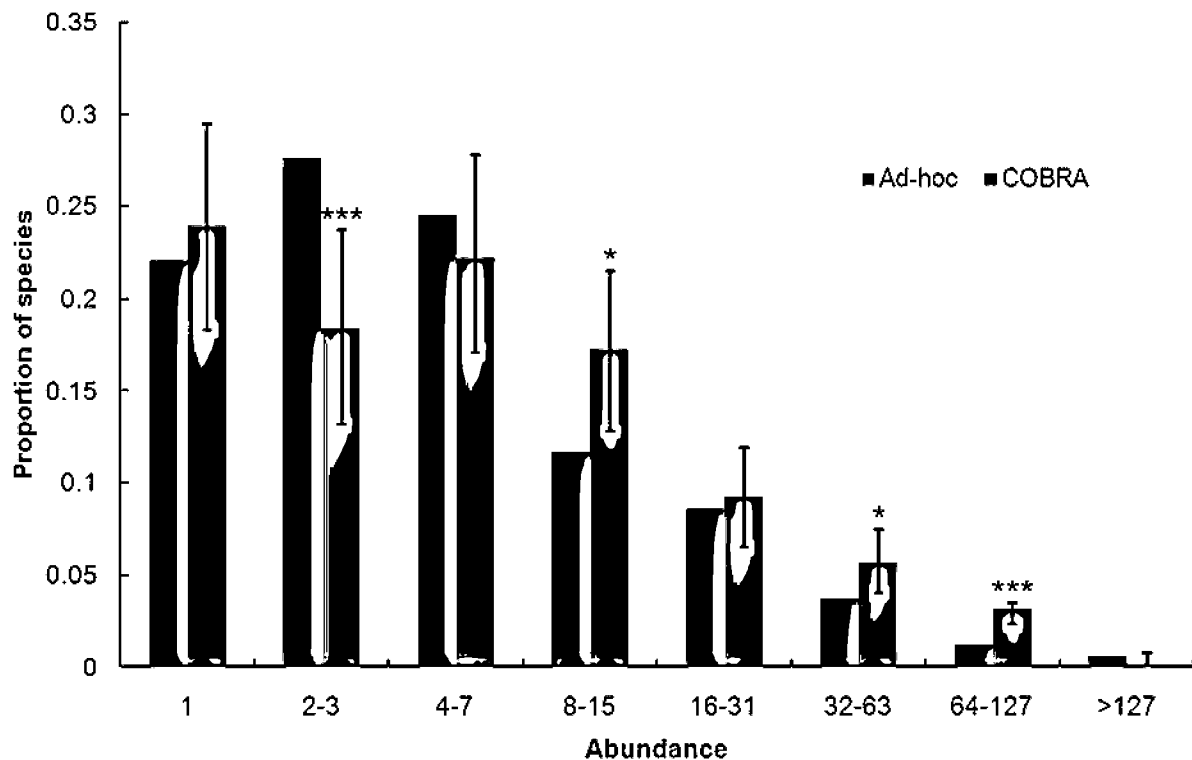
The proportion of singletons was not found to be statistically different (*Ad-hoc* = 0.221; COBRA = 0.240; $p = 0.486$). Although *ad-hoc* samplers did intentionally look for new individuals of species for which only one specimen was collected, the differences between approaches are not significant. As the COBRA used complementary sampling areas and methods, the number of spatial and methodological edge effects (as defined by [10,27]) is probably much reduced compared to any protocol that would use a single method in a single site. Most singletons in high diversity sites can be explained by low sampling effort [28]. As long as the overall effort reaches a minimum, the number of singletons can be much reduced, even with protocols that do not explicitly aim at this reduction.

2.5. Species Abundance Distribution

The species sampled by both approaches are divided by abundance classes in different ways (Figure 2). The *ad-hoc* approach reveals a unimodal pattern with the octave for species with two and three individuals being dominant and few species having high abundances. The COBRA sampling reveals a bimodal pattern with singletons and species with between four and seven individuals being the most represented. It also includes many species with high abundances. The second abundance class, representing species with two or three individuals, therefore has a significantly higher proportion of species for the *ad-hoc* sampling. Conversely, most of the high abundance classes present significantly higher proportions of species for the standardized protocol.

The differences found between approaches reveal that the way individuals are apparently distributed by species is strongly dependent on sampling artifacts. One or none of the approaches accurately reflects relative species abundances in the community. The reasons behind such dependency in sampling approach are varied and related to the differences in efficiency. While a collector doing *ad-hoc* sampling may avoid collecting species that have already been sampled in reasonable numbers, a standardized sampling is unbiased in this respect. Hence the larger number of abundant species in the latter (Figure 2) [27-29]. On the other hand, *ad-hoc* samplers also tried to intentionally avoid singletons by looking for new individuals of such species. Hence the higher proportion of the second abundance class for *ad-hoc* sampling (Figure 2).

Figure 2. Abundance classes (octaves) for the species communities sampled by the *ad-hoc* and COBRA approaches. The latter was rarefied to the same number of individuals of the *ad-hoc* sampling and its 95% confidence limits are indicated, as are the significance levels of comparing the two approaches (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).



2.6. Average Specimen Size

Contrary to predictions, the average size of captured specimens was significantly larger for the standardized sampling (*Ad-hoc* = 5.191 mm; COBRA = 5.645 mm; $p < 0.001$). This was surprising as the *ad-hoc* sampling heavily relied on spotting conspicuous species and this may cause severe biases towards some taxa [30]. However, two factors may be playing an important role in this pattern. Firstly, the *ad-hoc* sampling included winter samples that largely contain linyphiids, some of the smallest and most abundant European spiders [21]. This family is not as common during Spring, when the standardized samples took place. Secondly, some species with higher abundances in the standardized samples were relatively large (*Phrurolithus minimus*, 218 individuals, 2.6 mm of average body size; *Trochosa ruricola*, 217, 9.7 mm; *Tetragnatha montana*, 206, 8.1 mm; *Kochiura aulica*, 141, 3 mm) compared with the most abundant species in the *ad-hoc* samples (*Zodarion atlanticum*, 142 individuals, 2.8 mm of average body size). The pattern found is therefore very dependent on the community structure sampled, masking any effects of sampling approaches.

2.7. Average Taxonomic Distinctness

The average taxonomic distinctness was similar for both approaches (*Ad-hoc* = 1.577; COBRA = 1.562; $p = 0.126$). As the explicit objective of both was to inventory the local spider diversity,

irrespective of any families or preferential taxa, results end up being similar. Apparently, either *ad-hoc* sampling or standardized sampling could be used to properly represent the community present at heterogeneous areas, with no evident taxonomic bias.

2.8. Behavior of Richness Estimators

According to the different algorithms, both approaches apparently sampled more than 50% of the species estimated to be present at the reserve (Table 1). However, the behavior of all algorithms is far from ideal, with none reaching the asymptote and all providing very different values (Figure 3). Also, the percentage of singletons is higher than 20% in all cases (Table 1) and the singletons and doubletons curves have not crossed by the end of the sampling process (Figure 3). This means that the richness should be much underestimated and in fact we cannot infer on the true richness of the sites, or their sampling completeness, with the existing algorithms. Accurate estimation of species richness with non-parametric estimators requires inventories close to completeness [28] and that was not the case. Both sampling approaches would have to be much more exhaustive, with a higher sampling intensity, to allow the true richness of the areas to be assessed.

However, in the case of the two spots sampled by the COBRA protocol, we could estimate beforehand that the effort and combination of methods applied should capture close to 50% of the species present at each site at the time of sampling [19]. The true richness of each spot should probably be at least 164 species at the riparian corridor and 188 species at the meadow, values substantially higher than the ones provided by the non-parametric estimators. Given the behavior of these, typical of underestimation, the given values should in fact be closer to the sites' true richness. These results emphasize the importance of having some kind of reference for what portion of the community is expected to be sampled before committing to a sampling protocol [19]. Any other approach, even if as efficient, would require the use of probably more unreliable estimators to assess the true richness of the site.

3. Experimental Section

3.1. Study Area

The study area was the Marsh of Arzila, located 11 km west of Coimbra, central Portugal, with the UTM coordinates of 29TNE38 (using 10×10 km squares), at an altitude of 25 m (Figure 4). This damp habitat comprises an area of about 150 ha ($3 \text{ km} \times 500 \text{ m}$), at the south margin of the Mondego River. The annual mean temperature is 15.3 °C, being the minimum mean temperature 4.9 °C in January and the maximum mean temperature 27.5 °C in August. The mean annual precipitation recorded is 891 mm and the mean annual relative air humidity is 75%. This area, currently classified as a Nature Reserve, was very close to a drainage process in 1980, under the Hydroagricultural Plan for the Lower Mondego; however, different studies [31,32] granted the current conservation status to the area.

Figure 3. Randomized accumulation curves for observed species richness, singletons, doubletons, and several different non-parametric species richness estimators.

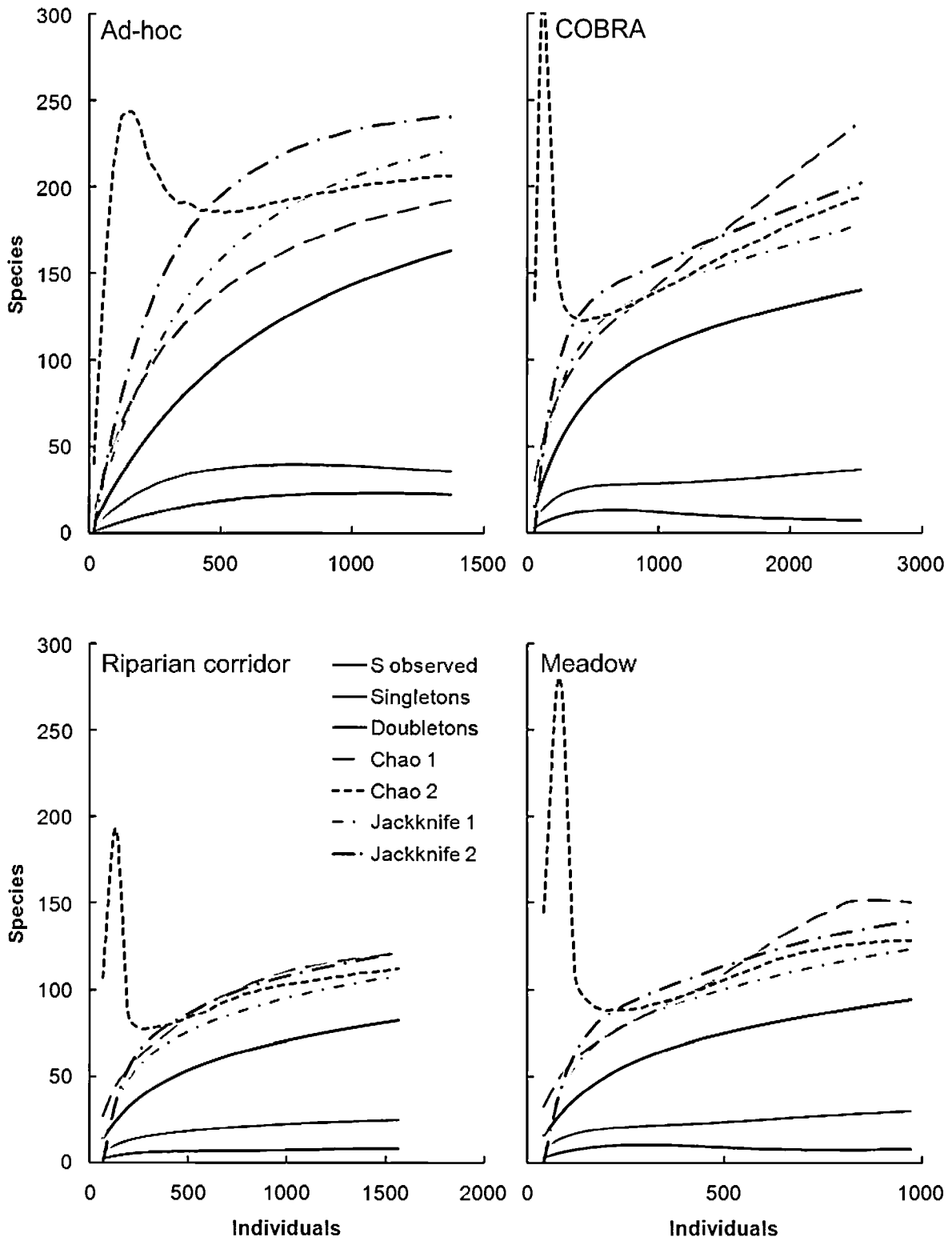
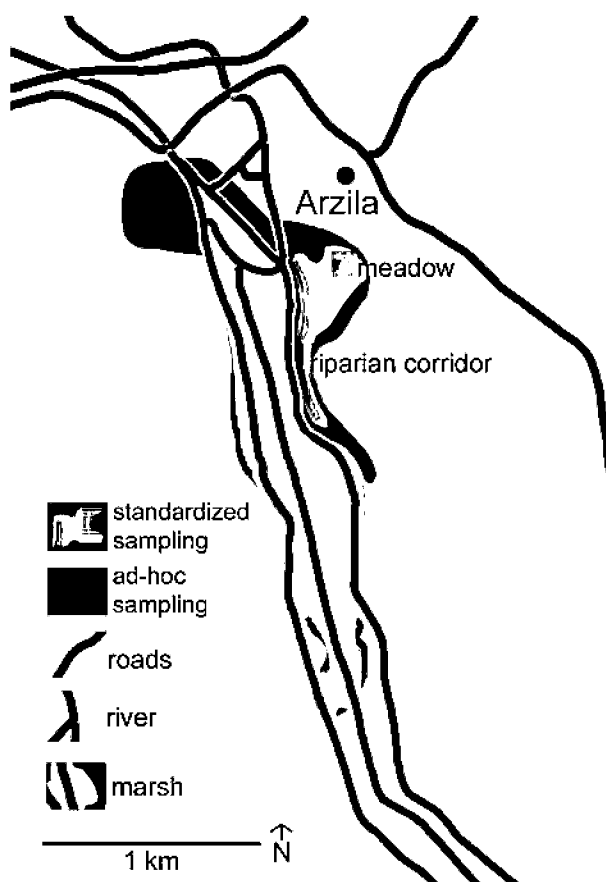


Figure 4. Schematic representation of the sampling area, the Marsh of Arzila, in Central Portugal. The two plots sampled with the standardized and optimized protocol COBRA are indicated, as is the area sampled *ad-hoc* during one and a half years.



3.2. *Ad-hoc* Sampling

This consisted in one and a half years of sampling, in a total 24 person-days, from October 23, 2005 to May 14, 2008 (with no samples during 2007), during different seasons, but mainly focusing on spring months. Effort was approximately distributed per season as: Spring, 50%; Summer, 12.5%; Autumn, 25%; Winter, 12.5%. Most of the *ad-hoc* samples were done by hand-collecting and sweeping net. Occasionally, pitfall trapping, tree beating and leaf litter examination was conducted. Effort was approximately distributed per method as: hand-collecting, 30%; sweeping, 40%; tree beating, 15%; pitfall trapping, 10%; searching in leaf litter, 5%. There were only three night sampling occasions (approximately 10%). The sampling area comprised the northern part of the reserve (Figure 4). In order to avoid the over-collection of common species, specimens were released in the field when recognizable as a previously collected species.

3.3. *Standardized and Optimized Sampling*

The optimization procedure required to optimize area, time and sampling methods. Optimizing the sampled area called for a minimum number of plots as dissimilar as possible in vegetation structure, to capture a maximum number of species with minimum effort. We have therefore chosen a riparian corridor and a meadow. The riparian corridor was 700 m long and about 10 m wide along most of its

length, surrounded by water on both sides. The main vegetation at the arboreal stratum was *Salix alba*, *Salix atrocinerea*, *Fraxinus angustifolia* and *Fraxinus excelsior*. The herbaceous stratum was mainly comprised by *Geranium purpureum*, *Urtica membranacea*, *Rubus ulmifolis*, *Arundo donax*, *Cirsium vulgare* and *Lythrum salicaria*. The meadow was about 80 m × 60 m, surrounded by several different plantations of *Pinus pinaster*, *Quercus faginea*, *Vitis vinifera* and *Eucalyptus globulus*. The main vegetation was *Holcus lanatus*, *Dittrichia viscosa*, *Lythrum junceum*, *Tolpis barbata* and *Andryala integrifolia*.

Optimizing the sampling season required all samples to be taken during the Spring species richness peak, from 11 to 28 of May, 2008 [21].

The optimization also required choosing the best possible combination of methods. Although a target of 80/90% of species captured and listed is usual for vertebrates, even at large scales [33,34], this requires a very large and often unreasonable effort for arthropods [6,8]. To each plot we have therefore applied the COBRA50 protocol [19]. This semi-quantitative protocol was optimized to capture close to 50% of the species present in each site, independently of the habitat, with minimum effort [19]. As predetermined by the protocol, we applied a different set of methods to each plot, according to the habitats in question and the vegetation layers that were present (Table 2). The overall number of samples was nevertheless the same for both plots (24). With this protocol, each person-day of work is equivalent to six person-hours, so that fatigue does not influence the results [19]. Therefore, each plot had the equivalent to four person-days of effort.

Table 2. Number of samples per method at each plot sampled with the standardized and optimized protocol [19]. Each sample is equivalent to one person-hour of collecting and a pitfall sample is made by 4 pooled traps (for an explanation of methods see [6,8,9]). Most methods were performed on May 11, 2008, except for pitfall traps which were left in the field from 14 to 28 of May, 2008.

Site	Riparian corridor	Meadow
Aerial sampling night	4	0
Beating day	2	0
Beating night	2	0
Ground sampling night	0	4
Sweeping day	2	4
Sweeping night	2	4
Pitfall traps	12	12
Total	24	24

3.4. Statistical Analyses

Testing the efficiency of increasing the observed species richness by unit of time (objective 1) required comparing both approaches (the *ad-hoc* sampling and the summed results for both COBRA plots) by means of accumulation curves drawn by person-days of fieldwork. Some authors argued that all comparisons of species richness between communities should be made with effort rescaled by individuals [35,36]. Although this is true for species richness comparisons, our goal was to compare

the effort required to attain certain richness levels and not to compare final values *per se*. In such case it is better to make comparisons with some direct measure of effort or standard sampling unit [33,37]. The curves and 95% confidence limits followed the Mao Tau values [38,39] as computed by the EstimateS software [40]. The efficiency by captured individuals (objective 2) was compared in the same way as the previous analysis but with curves resampled by the number of individuals.

To test if the COBRA would collect more specimens of the most abundant species than the *ad-hoc* sampling (objective 3) we compared them using the Simpson's measure of evenness [41]:

$$E_{1/D} = (1/D)/S \quad (1)$$

where S is the species richness and D is the Simpson index [42,43]:

$$D = \sum p_i^2 \quad (2)$$

where p_i is the proportion of individuals in each species. The evenness measure changes between 0 and 1, with maximum value when all species are equally abundant, and is independent of richness. However, in order to give statistical significance to the comparison between approaches, we had to rarefy the largest "community", as given by the COBRA protocol, with rarefaction and permutation tests [35,44]. The evenness value of the *ad-hoc* sampling was compared with the 95% confidence limits of the values calculated for the same number of individuals randomly chosen from the standardized sampling. The confidence limits were given by the 0.025 and 0.975 percentiles of all permutations. If the value for the smaller sample falls outside the limits of the larger sample then there is a significant difference between samples. Custom Java code was written to calculate the index and confidence limits for the COBRA protocol with 1,000 permutations (code available from the first author by request).

For comparing the proportion of singletons between the two approaches (objective 4) the same code used for the previous analysis was used to do 1,000 permutation tests, this time with the percentage of species represented by a single individual as the variable.

The species abundance distributions (objective 5) were also made after rarefaction of the COBRA protocol with 1,000 permutations so that it would be comparable with the *ad-hoc* sampling. Classes were determined by dividing species according to a log₂ division of octaves [43]. To guarantee that all octaves had twice the number of abundance values as the previous, the first octave included only the singletons. The second octave included all species with two or three individuals, with the following octaves having inclusive lower bounds of 4, 8, 16, 32, 64 and 128 individuals. Because the number of species captured by each approach was different, we compared the distributions based on the proportion of species falling into each abundance class. Finally, we calculated the 95% confidence limits for the COBRA protocol after rarefaction, and attributed significance values for each comparison of sampling approaches in the same abundance class.

To calculate the average size of captured specimens (objective 6) required that an average size was attributed to all species. This was based on different literature according to the family or genus. When males and females had different sizes, with males almost invariably presenting the smallest values, the average of both was calculated. The size of each specimen was assumed to be the average size of its species. For comparison of approaches, the same software used for the previous analyses was used once again to do 1,000 permutation tests, this time with the size of individuals as the variable.

The average taxonomic distinctness (avTD) (objective 7) was calculated based on the inclusion of species in families and genera [45]. The taxonomic distinctness between two species was considered to be 2 when they belonged to different families, 1 when they belonged to the same family but different genera and 0 when they had the same genus. Values for all possible pairwise comparisons between species were calculated for the *ad-hoc* approach and their average was calculated as the avTD [46,47]. For the standardized sampling, 1,000 permutations were done, always with the same number of individuals as the *ad-hoc* approach, randomly chosen from the larger sampling, and the avTD calculated for each of the permutations. Confidence limits could therefore be also calculated for avTD values.

The software package EstimateS [40] was used to calculate randomized species accumulation curves for the observed species richness (using the Mao Tau procedure), singleton and doubleton curves and various non-parametric richness estimators (Chao1, Chao2, first and second order Jackknife), using 1,000 randomizations in all calculations (objective 8). All curves were sample-based and rescaled to individuals [35]. The behavior of estimators was assessed according to their ability to asymptote and provide reliable values.

4. Conclusions

Both approaches, *ad-hoc* sampling and use of a standardized and optimized protocol may prove advantageous under different circumstances. However, in the case studied, the COBRA protocol revealed two main advantages: (1) it was more efficient when effort was measured by person-days of fieldwork; (2) it allowed a probably more reliable estimation of the true number of species present in each area at the time of sampling than using mostly unpredictable species richness estimators. An additional advantage is that (3) it allows meaningful comparisons between areas even when these are undersampled.

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