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Establishing reliable spider (Araneae, Araneidae and Thomisidae) assemblage sampling protocols: estimation of species richness, seasonal coverage and contribution of juvenile data to species richness and composition

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ABSTRACT

The capacity of short-term sampling to provide reliable estimates of annual spider assemblages (Araneidae and Thomisidae) present in a Mediterranean site was analyzed, along with the contribution of juvenile data on estimations of spider species richness. A standardized year-long sampling protocol in a one-square-kilometer plot in central Spain yielded reliable Araneidae and Thomisidae inventories. To compare sampling design efficiencies, the degree of completeness of collected annual inventories was estimated, along with an “optimal sampling” selection of months, i.e. the minimum number of months indicating most accurately the number of species present throughout the year. The completeness of spring-month sampling, as well as that of every month, was also estimated. Calculations both included and excluded immature stages. When multiple localities must be sampled and fieldwork minimized, a 1-month spring sampling protocol reasonably estimates the entire spring fauna, allowing effective comparisons between sites during the richest period. Our results indicate that juveniles must be included in the sample in order to obtain reliable estimates of species richness, and they should be stored apart from adults in order to analyze them separately as advances in their identification are achieved.

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1. Introduction

Although diversity patterns across taxa do not necessarily correlate (Reid, 1998; French, 1999; Kotze and Samways, 1999; Sætersdal et al., 2003), management and design of biodiversity conservation strategies are frequently based on information derived from some well-known taxa. It follows, then, that conservation policy in general could be enhanced by improving current knowledge of spatial biodiversity pat-

terns of those taxa, such as Arthropods, which account for the greatest part of biodiversity (Kremen et al., 1993); consequently, much more corroborative field survey work must be still done (Koch et al., 2000). However, reliable field sampling of such highly diverse taxa is no simple task. A long-term, intensive inventory, involving many sample sites in an extensive territory is often impossible for hyperdiverse groups, due to resource (mainly time and money) limitations. Hence, short-term sampling programs capable of reliably identifying all species present in a site are needed. To develop such programs, the seasonal dynamics of the studied assemblage must be well-known (e. g. Landau et al., 1999; Moreno and Halffter, 2000; Cardoso, 2004).

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Although for some taxa, short-term samplings have proved to be useful (e. g. ground beetles: Maelfait and Desender, 1990; moths: Landau et al., 1999), some authors have argued that a reduction in spider sampling effort should not imply a decrease in the seasonal width of the sampling protocol (Churchill and Arthur, 1999; Riecken, 1999). However, in Mediterranean areas, where summer heat and drought determine a bimodal arthropod species richness distribution, the species richness peaks in spring, and is followed by a smaller autumn peak that does not seem to add many new species (Shapiro, 1975; Urones and Puerto, 1988; Molina, 1989; Cardoso, 2004). Thus, the gathering of faunistic information from a variety of Mediterranean sites most quickly and efficiently would require the examination of the reliability of short-term sampling design estimations of total species numbers and their comparison with that which could otherwise be obtained over the course of an entire year. We did this by studying the Araneidae and Thomisidae assemblages in a Mediterranean site over a complete annual life cycle.

Another important feature of arthropod sampling, especially when dealing with spiders, is the treatment of immature stages. Usually juveniles are discarded in spider biodiversity studies (e.g. Jerardino et al., 1991; Toti et al., 2000; Sørensen et al., 2002) because they are difficult to identify (Coddington et al., 1996; Dobyns, 1997). However, some authors have kept undeveloped stages in the laboratory until maturity in order to include their number in their analysis (e. g. Urones and Puerto, 1988). As juvenile numbers may profoundly influence temporal and spatial spider biodiversity patterns, one must be careful when juveniles are used to compare assemblages and it has been suggested that they should be analyzed separately (Norris, 1999). However, the inclusion of juveniles seems to be necessary in order to obtain reliable short-term sampling estimates of whole-year species richness and composition (Toti et al., 2000; Scharff et al., 2003). Thus, as Coddington et al. (1996) recommended, we have also examined the effects of including juveniles on species richness estimations, in two families of an abundant non-specialist predator functional guild (Wise, 1993) in which the identification of juveniles is feasible (i.e. Araneidae and Thomisidae).

The aims of this study are:

- to analyze the capacity of several short-term sampling designs to provide reliable estimates of spiders diversity in a Mediterranean area and;
- to study the effect of juveniles on species richness estimations.

2. Methods

2.1. Study site

The study was carried out from April 2003 to March 2004 in a 1 km² site in central Spain, in the southeast of the Comunidad de Madrid (Perales de Tajuña, 40°14'25" N 3°23'38" W). The vegetation is at present dominated by kermes-oaks (*Quercus coccifera*), with a dense shrub undergrowth of *Rosmarinus officinalis* and *Stipa tenacissima*. This sampling site is at 600 m

elevation, with a Mediterranean climate and limestone substratum.

2.2. Sampling protocol

Two spider families, Araneidae and Thomisidae, have been studied. These families were selected due to the ease of identification of their juveniles (see below), and because accurate inventories could be obtained. Jiménez-Valverde and Lobo (2005) demonstrate that reliable inventories of these two taxonomic groups can be gathered in one-square kilometer Mediterranean sampling sites. Briefly, the 1 km² sampling plot was divided into 2500 subplots of 400 m²; 20 of these subplots were chosen at random, and a subsample unit carried out in each. A subsample unit was defined as: i) a one-person sweep of the herbaceous vegetation and shrub during 15 min, ii) a one-person beating of bushes and small trees and branches during 15 min, and iii) the running, during 48 hours, of 4 pitfall traps 11.5 cm wide and 1 l in volume, each separated by 10 m from the others. Traps were filled with water, and a few drops of detergent added to break the surface tension so as to prevent the spiders from escaping. Sampling was always done by the same person (A.J.-V.) in order to avoid possible differences due to the effect of the collector (Norris, 1999). Rainy and windy days were avoided in order to prevent a reduction in the efficiency of the sampling methods. This protocol gathers reliable Araneidae and Thomisidae inventories and is highly repeatable because the data gathered are related to sampling effort measure (see details in Jiménez-Valverde and Lobo, 2005). This protocol was performed once a month (except in April, when it was done twice) in order to study the seasonal variation of the richness and composition of the assemblage (dates of sampling: Ap-1: 1/IV/03-9/IV/03, Ap-2: 22/IV/03-29/IV/03, Ma-Ju: 31/V/03-6/VI/03, July: 16/VII/03-23/VII/03, Aug: 13/VIII/03-26/VIII/03, Sep: 17/IX/03-26/IX/03, Oct-Nov: 22/X/03-14/XI/03, Dec: 2/XII/03-12/XII/03, Jan: 9/I/04-21/I/04, Feb: 11/II/04-1/III/04, Mar: 10/III/04-t22/III/04). In total, 220 subsample units were carried out.

2.2.1. Juvenile sampling

Juveniles that could be identified to the species level were included in the analysis. This was possible for many araneid and some thomisid species which have a distinguishing, characteristic color pattern, and for some genera represented by only one species in the Iberian Peninsula (i.e. *Mangora acalypha* (Walckenaer, 1802), *Runcinia grammica* (Koch, C.L., 1837) and *Synaema globosum* (Fabricius, 1775)).

2.3. Data analysis

The degree of completeness of the collected annual inventory was estimated, as well as an "optimal sampling" selection of months, a minimum, to identify the year-round number of species. The completeness of the sample gathered during the spring months, as well as that of every month, was also estimated by means of i) species accumulation curves, ii) non-parametric estimators, and iii) the estimated area under the truncated lognormal species-abundance distribution curve.

Species accumulation curves were built (Gotelli and Colwell, 2001) using three sampling effort surrogates, which

are the number of individuals (N), the number subsamples (S), and the number of months (M; used only for the yearly sampling). However, the accuracy of monthly samplings was evaluated by using only the number of subsamples units (i.e. the number of subplots, 20 every month). The order in which sampling effort units were added was randomized 500 times to build smoothed curves using the EstimateS 5.0.1 software (Colwell, 1997). The asymptotic value of the accumulation curves obtained was estimated using both the Clench and Weibull equations (Soberón and Llorente, 1993; Colwell and Coddington, 1994; Flather, 1996; León-Cortés et al., 1998; Peterson and Slade, 1998). These models were fitted to the data through non-linear regression using the Simplex & Quasi-Newton algorithm (StatSoft, 2003).

Four non-parametric species richness estimators were calculated: the first- and second-order jackknife (Jack1 and Jack2), the abundance-based coverage (ACE) and the incidence-based coverage estimator (ICE). These four estimators have performed relatively well in numerous studies (Palmer, 1990; Palmer, 1991; Coddington et al., 1996; Boulinier et al., 1998; Toti et al., 2000; Walther and Martin, 2001; Borges and Brown, 2003; Brose et al., 2003; Chiarucci et al., 2003). Detailed descriptions of the estimators can be found in Colwell and Coddington (1994) and Colwell (1997).

The truncated lognormal distribution model was fitted to the abundance data (Magurran, 1988) in order to estimate the area under the curve, or the total number of species that could be expected if an exhaustive collection effort was carried out (Fagan and Kareiva, 1997). Octaves were defined as \log_2 following Preston (1948, 1962) (see Lobo and Favila, 1999) and the Kolmogorov–Smirnov one-sample test with Lilliefors corrected critical values was used to compare the observed and expected patterns of species abundance distributions (Tokeshi, 1993).

In order to cluster monthly inventories according to their taxonomic resemblance, the Sørensen similarity coefficient was used, taking into account presence/absence data. Because true absences of species are difficult to verify in inventories, this coefficient was selected because it doubles the weight of double presences (Legendre and Legendre, 1998). The Bray–Curtis coefficient (quantitative version of the Sørensen coefficient, Legendre and Legendre, 1998) was also used when considering abundance data. Because both cluster analyses generate similar dendrograms, only the results from the Bray–Curtis coefficient are shown. Ward's method was used as linkage rule, a method which tries to minimize the difference between the sum of the squared distances of cases and the mean values of the clusters to which they are assigned (Legendre and Legendre, 1998). We used NTSYSpc 2.11 (Rohlf, 2000) and STATISTICA (StatSoft, 2003) software in these analyses.

3. Results

3.1. Faunistic composition

A total of 1599 individuals were captured, 1471 of them juveniles (92%); of these, 1241 (84%) were used in the analysis, as the rest (230) were impossible to identify unambiguously to

species level. In all, 20 species were collected; three of them captured only as immature stages (*Aculepira armida* (Audouin, 1826), *Gibbaranea* sp. Archer, 1951 and *Hypososinga albovittata* (Westring, 1851)).

3.1.2. Seasonal variation of the assemblage

The seasonal number of both species and individuals from adult + juvenile data peaked in spring (Ap-1, Ap-2 and Ma-Ju). Numbers decreased in summer (Jul, Aug and Sep), while a nearly constant recovery occurs afterwards (Fig. 1A). Interestingly, the number of species was quite stable from March to May–June, unlike the number of individuals. On the other hand, in the case of adults-only data, a clearly defined peak both in the number of species and individuals occurred in late spring (Ap-2 and Ma-Ju), followed by a sharp decline that persists throughout the rest of the year (Fig. 1B). In the case of adults-only data, the seasonal variation in the number of species was more erratic, as there were months in which no

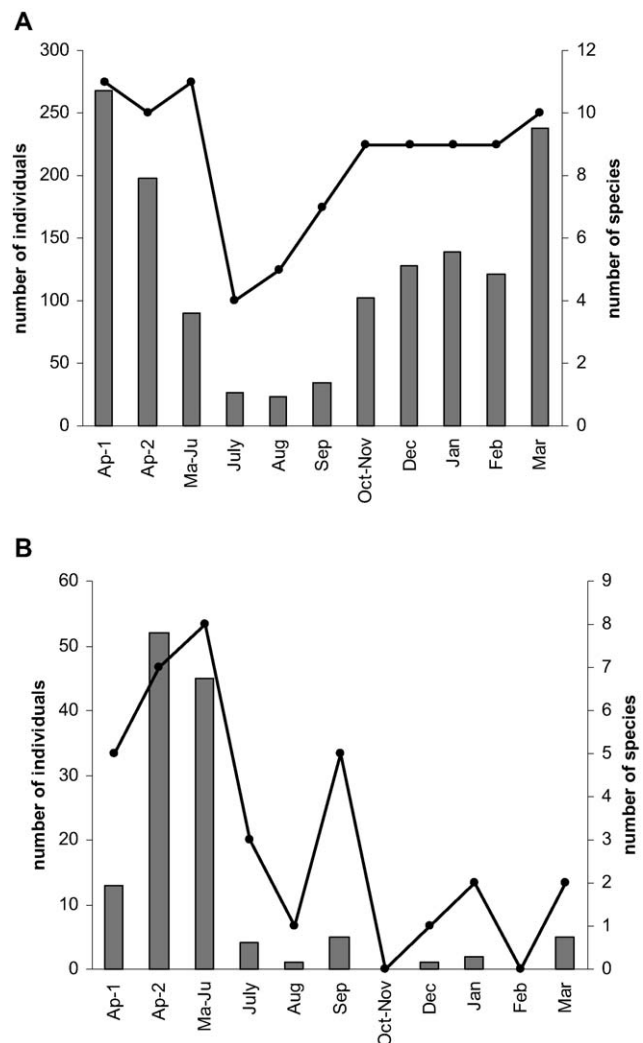


Fig. 1 – Seasonal variation of the number of species (lines) and number of individuals (columns) of the spider assemblage studied including juveniles (A) and adults-only specimens (B).

adult spider was collected, as in October–November or February.

Months grouped according to faunistic similarity, and which include juveniles, belonged to either a group that contains the less-species-rich summer inventories, with fewer individuals (Jul, Aug and Sep), or to another with the remaining inventories (Fig. 2A), in which the composition of the fauna identified in the species-rich months was very similar. Adults-only data generated a similar tree diagram, in which species-rich spring inventories are again clearly associated (Fig. 2B).

3.2. Accuracy of the annual sampling

Lognormal estimations, accumulation curve functions and non-parametric estimators all showed that observed richness represented around 80% of that estimated when both adult and juvenile data were considered (Table 1). Accumulation curves nearly reached an asymptote (Fig. 3); the esti-

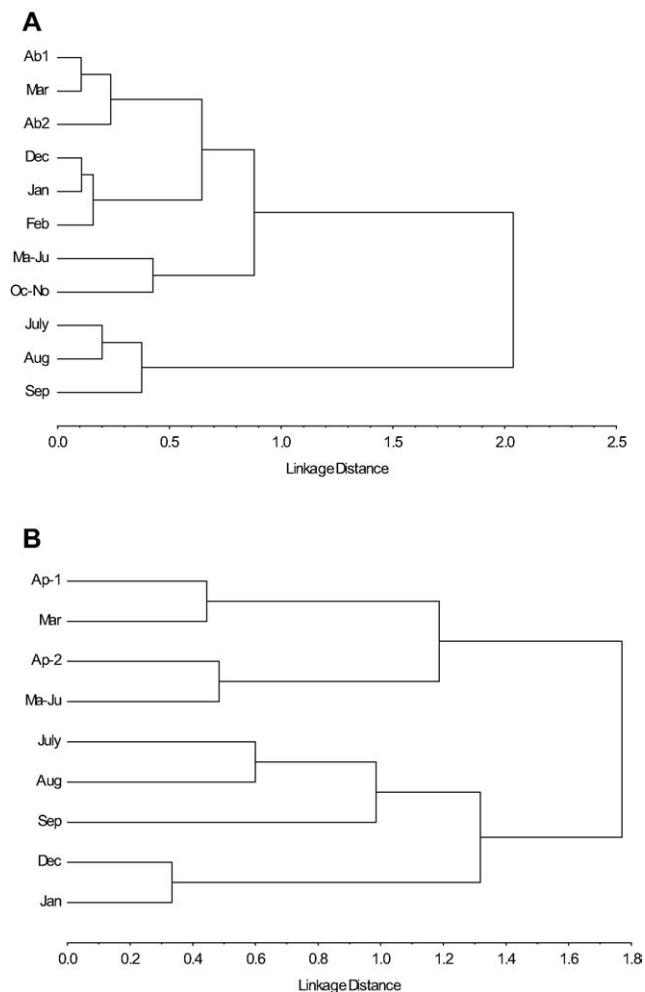


Fig. 2 – Dendrograms showing the similarity of monthly samplings using the Bray-Curtis similarity coefficient as resemblance measure and Ward’s method as linkage rule (A: juveniles included; B: juveniles excluded). Note that no adults were captures neither in Oct–Nov nor in Feb, so such samples are excluded from dendrogram B.

mates by the various methods suggest that only about three to five more species would be collected if an exhaustive sampling effort was carried out. Adults-only data lead to higher estimation scores (Mann–Whitney U test = 2.63, $P = 0.008$) and also to a greater range of variation (26.5 ± 1.2 species; mean \pm S.E.) than those obtained from all-specimens data (22.7 ± 0.3 species). This adults-only data leads to an observed species richness of around 60% of estimated species richness, and would indicate that almost 10 more species should have to be added to the inventory (Table 1). However, a clear pattern in the variation in the level of completeness, according to the measure of sampling effort used (subsamples, months or individuals; Table 1), was not apparent.

Adult + juvenile species abundance data closely fits a lognormal truncated distribution ($D = 0.051$, ns), as well as the species abundance distribution of adults ($D = 0.063$, ns), indicating a very similar total number of species (24 or 25 species; see Table 1).

Non-parametric estimators with adults-only data tended to overestimate species richness and lead to considerably different predictions of species richness. As adults-only data produced a greater proportion of rare species (singletons and doubletons) (Table 1 and Fig. 3), the inventories so derived seemed, a priori, to be more incomplete than those derived from all-specimens data. Moreover, Clench and Weibull estimations were affected by the difference in the shapes of the accumulation curves (see Fig. 3). A high percentage of singletons in the data resulted in an extremely gradual rate of species addition, leading to steeper accumulation curves and greater function slopes at the end of the curves. The Clench adults-only final slopes range from 0.7 to 0.03; all-specimen final slopes from 0.2 to 0.001; Weibull adults-only final slopes range from 0.6 to 0.02; all-specimen final slopes from 0.3 to 0.002. Thus, adults-only data clearly lead to overestimations of species richness, especially in the case of the Weibull model (Table 1).

Clench and Weibull models extrapolated to values of even twice the number of individuals actually collected yield curves that were still non-asymptotic (especially the latter; Fig. 4). This indicates that doubling the sampling effort would not make the collected inventories appreciably more representative. The “reasonably true” species richness was therefore chosen as 24, for purposes of comparison.

3.3. Accuracy of the optimal sampling

By an iterative procedure, months were selected sequentially, according to their contribution to the total accumulated species richness, until no more new species were added (Table 2). A seasonal optimal sampling selection indicates that, in accordance with all-specimen data, four months is the minimum period necessary to guarantee the capture of a number of species equal to that collected in the year-long sampling. If juveniles are not included, five months are necessary. Three sampling periods (Ap-1, Ma-Ju and Sep) were found to be common to the results from both data sets. A random selection (500 randomizations) of the same number of months produced a significantly smaller number of observed species (Fig. 3).

Table 1 – Sampling design results, with and without juveniles (A + J, A) and calculated for various sampling effort units (subsamples, months and individuals): number of observations or sampling units, number of species observed (Sobs), number and percentage over Sobs of singletons (species with only one individual) and doubletons (species with only two individuals), number of species predicted (Spred) and percentage over Sobs of the Clench and Weibull functions, number of species predicted and percentage over Sobs of the ICE (incidence-based coverage), ACE (abundance base coverage), Jack1 (first-order Jackknife) and Jack2 (second-order Jackknife) nonparametric estimators, and number of species predicted and percentage over Sobs by the lognormal abundance distribution

	Complete sampling						Optimal sampling				Spring sampling			
	A + J Subsam- ples	A + J Months	A Subsam- ples	A Months	A + J Indivi- duals	A Indivi- duals	A + J Subsam- ples	A Subsam- ples	A + J Indivi- duals	A Indivi- duals	A + J Subsam- ples	A Subsam- ples	A + J Indivi- duals	A Indivi- duals
Number of observations	220	11	220	11	1369	128	80	100	514	68	80	80	794	115
Sobs	20	20	17	17	20	17	20	17	20	17	15	10	15	10
Singletons	3	3	7	7	3	7	7	10	7	10	1	2	1	2
%	15	15	41.2	41.2	15.0	41.2	35.0	58.8	35.0	58.8	6.7	20.0	6.7	20.0
Doubletons	3	3	3	3	3	3	3	1	3	1	1	1	1	1
%	15	15	17.6	17.6	15.0	17.6	15.0	5.9	15.0	5.9	6.7	10.0	6.7	10.0
Spred Clench fit	21.2	23.5	22.2	31.9	20.9	21.6	22.6	23.4	22.0	23.3	16.0	11.3	15.6	10.9
%	94.2	85.2	76.4	53.3	95.7	78.8	88.6	72.7	90.9	72.8	93.9	88.8	95.8	91.3
Spred Weibull fit	24.3	22.6	29.1	22.1	24.8	39.3	33.3	46.0	54.4	29.0	15.9	10.2	16.2	10.4
%	82.4	88.4	58.5	77.1	80.5	43.2	60.0	36.9	36.8	58.7	94.5	97.9	92.5	96.5
ICE	22.4	22.1	27.4	23.2	-	-	31.1	35.3	-	-	15.4	11.2	-	-
%	89.4	90.5	61.9	73.1	-	-	64.2	48.1	-	-	97.3	89.2	-	-
ACE	22.3	22.3	28.6	28.6	-	-	34.5	34.8	-	-	15.4	11.3	-	-
%	89.8	89.8	59.4	59.4	-	-	57.9	48.8	-	-	97.1	88.5	-	-
Jack1	23.0	22.7	24.0	23.4	-	-	26.9	26.9	-	-	16.0	12.0	-	-
%	87.0	88.0	70.9	72.8	-	-	74.3	63.2	-	-	93.8	83.5	-	-
Jack2	23.0	20.8	27.9	23.9	-	-	30.8	35.7	-	-	16.0	13.0	-	-
%	87.0	96.3	60.8	71.0	-	-	64.8	47.6	-	-	93.7	77.2	-	-
Lognormal estimation	-	-	-	-	24.4	25.0	-	-	37.9	27	-	-	16.2	10.5
%	-	-	-	-	82.0	68.1	-	-	52.8	63.0	-	-	92.6	95.2

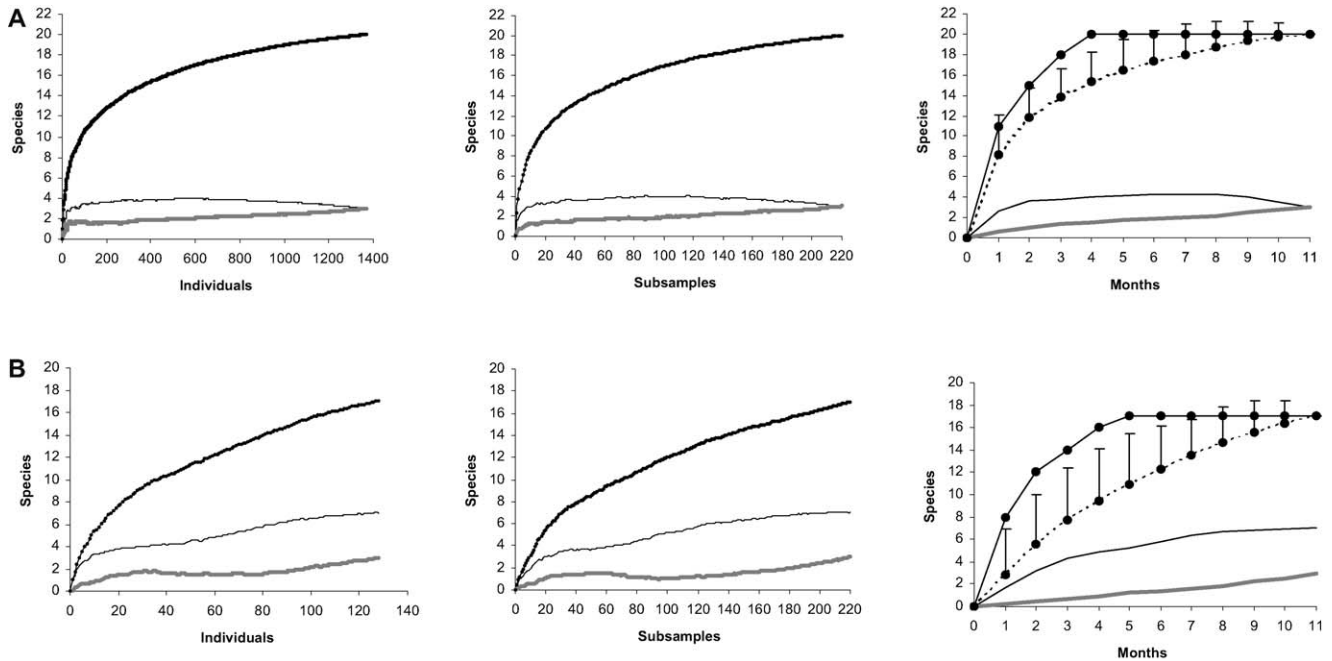


Fig. 3 – Randomized accumulation curves (500 times) for the annual inventory employing different sampling effort measures (A: juveniles included; B: juveniles excluded; thick line: species observed; thin line: number of singletons; gray line: number of doubletons). For the curve using the number of months as sampling effort measure the +95% confidence intervals have been indicated for the randomized curve, as well as the curve produced by ordering months according to a complementary criterion (“optimal sampling”, solid line).

Estimates derived from this optimal sampling period which include juvenile data were significantly higher than those from year-long periods (Mann-Whitney U test = 19.5, $P = 0.004$) but not from adults-only data which excluded juveniles (Mann-Whitney U test = 41.0, $P = 0.1$); the ranges of variation in the optimal sampling period are also higher (adults + juveniles 32.6 ± 3.2 , adults 31.3 ± 2.5). This means that there are 13 or 14 more species estimated than observed (Table 1). Thus, data from an optimal month selection operated on by predictive methods produces higher and more erratic species richness estimations than reasonably true scores. Moreover, accumulation curves did not approach an asymptote, especially when derived from adults-only data. Singletons and doubletons accounted for a higher proportion of species numbers than in the annual sampling (Table 1); the steep slope and proportion of singletons in adults-only data is especially remarkable, leading to some strikingly disproportionate estimates in the cases of the Weibull function and non-parametric estimators. Although species-abundance data closely fitted a lognormal distribution (adults + juveniles: $D = 0.09$, ns; adults: $D = 0.10$, ns), this method also overestimated the year-round number of species. Thus, the estimations generated with the inventory obtained from an optimal sampling procedure such as this generally produce overestimations of the true year-round species richness. Only the estimates generated by the Clench model, using both subsamples and individuals, yielded reasonable values (see Table 1).

3.4. Spring sampling

According to both species richness and compositional variation (Figs. 1 and 2) spring sampling was taken to be the faunistically similar period within which the annual species richness peaked (Ap 1 + Ap 2 + Ma-Ju + Mar). Accumulation curve functions, non-parametric and lognormal estimates (adults + juveniles: $D = 0.06$, ns; adults: $D = 0.10$, ns) all indicated that observed richness represented around 95% of that estimated from all-specimen data (Table 1). Estimates from adults-only data were slightly less stable, falling below the observed all-specimen richness. Clearly, excluding juveniles leads to an underestimation of spring species richness, which, at 16, would be considered to be its reasonably true value. However, this low value represents, respectively, only 80% and 67% of the observed and estimated annual species richness.

3.5. Monthly samplings

Adults-only data lead to underestimates of species in some months (Table 3). Moreover, there were some months in which only juveniles were collected (October-November and February). On average, five species (around 50%) were absent in spring months, juveniles excluded, while almost six species must be so considered (77% of species richness) in the remaining months. Thus, because excluding juveniles leads

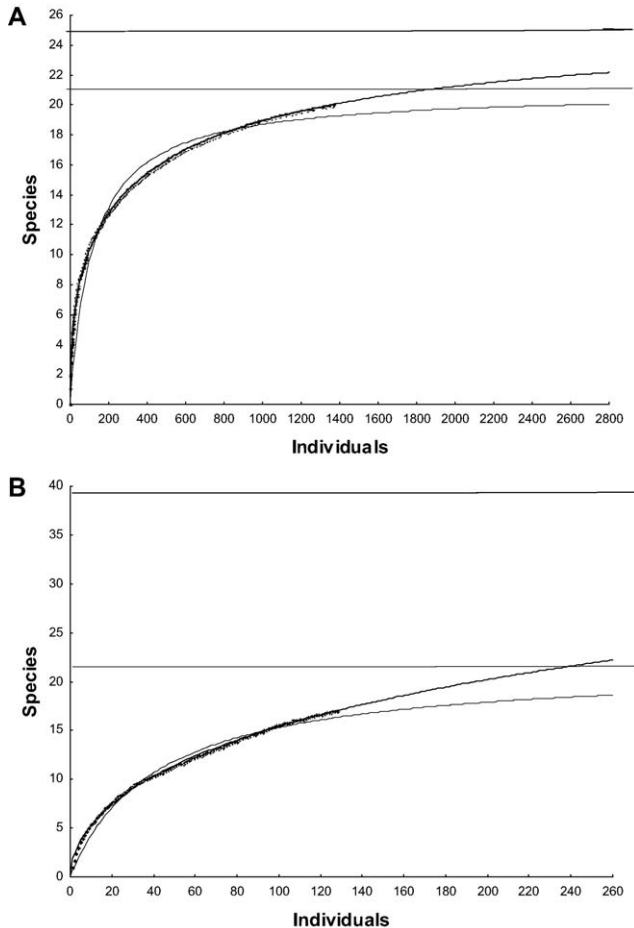


Fig. 4 – Extrapolations of the Clench and Weibull models by doubling the annual number of individuals actually collected (dotted curve: Clench model; solid curve: Weibull model; dotted horizontal line: Clench's asymptote; solid horizontal line: Weibull's asymptote). A: considering juveniles; B: excluding juveniles.

to underestimates of species richness values, only data that included juveniles were analyzed.

In general, monthly samplings were accurate, judging from a completeness of monthly inventories that varied around a value of 80% (Table 3, Fig. 5). The exceptions were inventories characterized by a high proportion of singletons from three months (August, February and, specially, September), which produced much steeper accumulation curves than those found for the other months (Table 3). The negative correlation of mean completeness with the standard deviation of estimates (*Spearman rank coefficient* = -0.80, $P < 0.01$) indicates that the more incomplete an inventory, the poorer the concordance (and so, accuracy) of estimators.

A single-month sampling in spring captured around 70% of the observed spring fauna, and around 50% of the year-round fauna (Table 3). Observed spring species richness varied between 62% and 69% of the estimated reasonable true spring spider species richness (16 species), and between 42% and 46% of the yearly estimated richness (24 species). More-

Table 2 – Results of an iterative complementarity procedure in which the inventories of each month were sequentially selected according to its contribution to the species richness

Adults + juveniles			
Iteration	Month	Species	Accumulated species
1	April 1	11	11
2	September	4	15
3	February	3	18
4	May-June	2	20
Adults			
Iteration	Month	Species	Accumulated species
1	May-June	8	8
2	September	4	12
3	April 1	2	14
4	July	2	16
5	December	1	17

over, monthly spring estimations varied from 62% to 100% of the estimated true spring species richness, and from 42% to 67% of the estimated true yearly species richness. However, estimations from both months and estimators varied greatly in their reliability; March was the least reliable of spring months, while the Jack2 estimator tends to produce an estimation extremely similar to the reasonably true spring species richness score (16 species, see above) (Table 3).

3.6. Similarity among sampling protocols

A gradient in Bray Curtis faunistic dissimilarity is observed from spring sampling, the more similar to the year-long sampling, to non-spring months (Fig. 6). The gradient in species richness similarity shows the same pattern with the exception of the optimal sampling which, obviously, marks the same species richness as the year-long sampling.

3.7. The effect of including juveniles

For purposes of comparison, the accumulated number of species using adults-only and adults + juveniles data were plotted together against number of subsamples (Fig. 7A). Not including juveniles produced lower observed species richness scores at every stage of the sampling process, increasing the difference as long as the sampling effort raised until an inflexion point (~70 subsamples) after which the advantage of including juveniles progressively disappeared (Fig. 7A). But, is the favorable effect of including juveniles attributable to the increase in sample size or to an intrinsic property of juveniles? We resampled 20 times the 1369 annual individuals (adults and juveniles) at $N = 128$ (the number of annual adults) calculating the number of observed species and estimating Clench predictions in order to detect the possible effect of sample size. The resulting observed scores varied from 9 to 14 (mean number \pm S.D.: 10.9 ± 1.4), while the estimations varied from 9.7 to 17.5 (13.2 ± 2.1). These values are significantly lower than those using the 128 adults as well as the 1369 adults and juveniles (see Table 1). A comparative examination of adult and adult-juvenile individual-based accumulation curves clearly shows the same pattern; i.e. that the same number of individuals

Table 3 – Results for the monthly samplings, with and without juveniles (because not including immature stages leads to a very low sample size, no estimator has been calculated in this case, see text): number of individuals collected, number of species observed (Sobs), number and percentage over Sobs of singletons and doubletons, number of species predicted (Spred) and percentage over Sobs of the Clench and Weibull functions, number of species predicted and percentage over Sobs of the ICE, ACE, Jack1 and Jack2 nonparametric estimators, and percentage of species observed over the total observed and predicted for spring and the annual sampling

Adults + juveniles	April 1	April 2	May–June	July	August	Sep-tember	Octo-ber–No- vember	De- cember	Janu- ary	Febru- ary	March
Number of Individuals	268	198	90	27	23	35	102	128	139	121	238
Sobs	11	10	11	4	5	7	9	9	9	9	10
Singletons	3 (27.3%)	1 (10.0%)	3 (27.3%)	1 (25.0%)	3 (60.0%)	5 (71.4%)	2 (22.2%)	2 (22.2%)	2 (22.2%)	5 (55.6)	2 (20.0%)
Doubletons	1 (9.1%)	0	0	1 (25.0%)	1 (20.0%)	0	2 (22.2%)	1 (11.1%)	0	0	1 (10.0%)
Spred Clench fit	12 (91.7%)	11 (90.9%)	12 (91.7%)	5.1 (78.4)	7.94 (63.0%)	11 (63.6%)	12 (75.0%)	11 (81.8%)	10 (90.0%)	12 (75.0%)	12 (83.3%)
Spred Weibull fit	14 (78.6%)	13 (76.9%)	12 (91.7%)	4.66 (85.8%)	11.24 (44.5%)	253 (2.8%)	11 (81.8%)	10 (90.0%)	9 (100%)	230 (3.9%)	13 (76.9)
ICE	14 (78.6%)	13 (76.9%)	13 (84.6%)	5.09 (78.6%)	12.03 (41.6%)	30 (23.3%)	11 (81.8%)	10 (90.0%)	11 (81.8%)	19 (47.4%)	11 (90.9%)
ACE	15 (73.3%)	10 (100%)	13 (84.6%)	4.69 (85.3%)	11 (45.4%)	31 (22.6%)	11 (81.8%)	11 (81.8%)	10 (90.0%)	20 (45.0%)	12 (83.3%)
Jack1	14 (78.6%)	13 (76.9%)	14 (78.6%)	4.95 (80.8%)	7.85 (63.7%)	12 (58.3%)	11 (81.8%)	12 (75.0%)	12 (75.0%)	14 (64.3%)	12 (83.3%)
Jack2	16 (68.7%)	16 (62.5%)	16 (68.7%)	5 (80.0%)	9.7 (51.5%)	16 (43.7%)	10 (90.0%)	12 (75.0%)	12 (75.0%)	18 (50.0%)	12 (83.3%)
S observed spring %	73.3	66.7	73.3	26.7	33.3	46.7	60.0	60.0	60.0	60.0	66.7
S observed total %	55.0	50.0	55.0	20.0	25.0	35.0	45.0	45.0	45.0	45.0	50.0
S predicted spring %	68.8	62.5	68.8	25.0	31.3	43.8	56.3	56.3	56.3	56.3	62.5
S predicted total %	45.8	41.7	45.8	16.7	20.8	29.2	37.5	37.5	37.5	37.5	41.7
Adults											
Number of Indi- viduals	13	52	45	4	1	5	0	1	2	0	5
Sobs	5	7	8	3	1	5	0	1	2	0	2
Singletons	3 (60.0%)	2 (28.6%)	3 (37.5%)	2 (66.7%)	1 (100%)	5 (100%)	–	1 (100%)	2 (100%)	–	0
Doubletons	1 (20.0%)	0	0	1 (33.3%)	–	0	–	–	0	–	1 (50%)

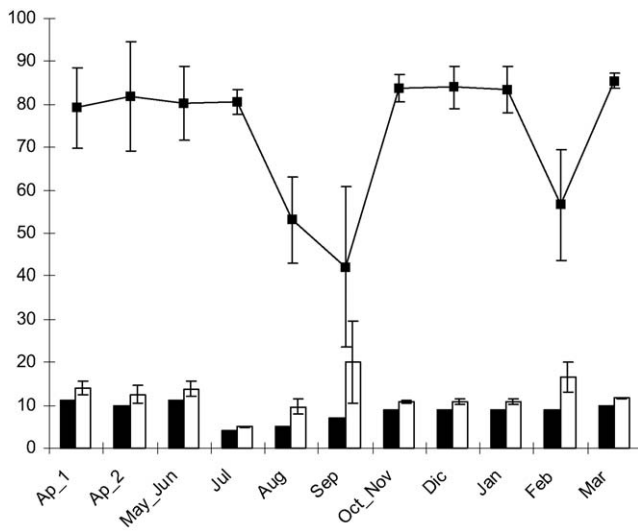


Fig. 5 – Number of observed species (black columns), mean of estimations ± S.D. (white columns) and mean percentage of completeness ± S.D. (square dots) over the annual total of species. All calculations made ignoring Weibull scores because of extremely high values (see Table 3).

yielded lower observed and predicted species richness scores using juveniles data than when only adults are used (Fig. 7B).

4. Discussion

The year-round survey carried out for this study yielded a good representation of the spider fauna of this kermes-oak forest. The seasonal pattern observed is typical of Mediterranean habitats where summer ushers in adversely high temperatures and pronounced drought (Shapiro, 1975; Abraham, 1983). In such conditions species richness peaks twice. One peak occurs in spring, when a maximum number of species is attained. Many juveniles appear at the beginning of this season, but only a small fraction reach maturity in May-June, when the adult peak of species richness is reached. In July there is a considerable decline in species and individual numbers, although later some new species appear in very small numbers during August and September. This population fluctuation is reflected in the similarity of spring and year-round assemblage structure (Fig. 6). Juveniles of the spring species appear again in autumn. Indeed, cluster analysis of data that included juveniles lead to a clear association of all monthly inventories except during July, August

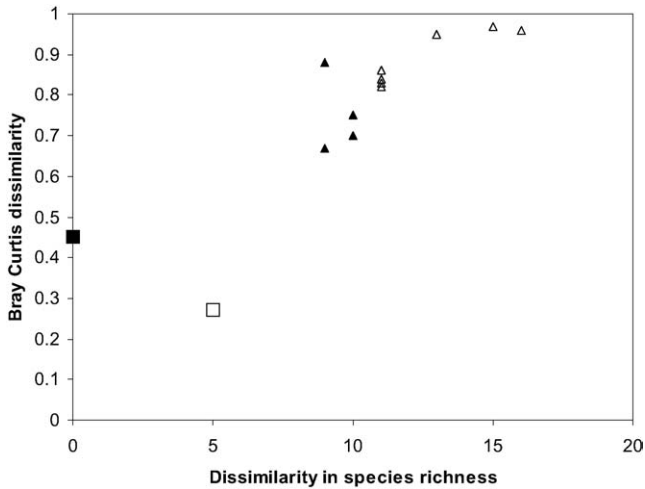


Fig. 6 – Difference between the number of annual collected species (20 species) and the collected richness in different inventories (i.e. dissimilarity in richness scores; X axis), and Bray Curtis faunistic dissimilarity distance (Y axis) of the different sampling protocols with respect to the year-long one (black square: optimal sampling, white square: spring sampling, black triangles: spring sampling months, white triangles: rest of months).

and September. However, adults-only data produced a separate cluster in winter months (December and January). The existence of these two phenologic peaks in species richness enhances the opportunities to find an effective short-term sampling design in order to reach a trade-off between reliability of inventories and survey costs.

Unfortunately, long-term intensive sampling may not be affordable in biodiversity surveys, especially those with multiple sampling points. A shorter-length optimal sampling protocol that collected the same number of species as year-round sampling would reduce the sampling effort. The main

problem with this strategy is that it significantly increases the proportion of singletons and the steepness of the accumulation curves, generating a sample with a species-abundance relationship different to that of the observed in the year-round assemblage (Fig. 6), biasing the estimations. Moreover, with many sampling locations, such an optimal protocol is still difficult to carry out.

Another strategy that would similarly reduce the required field effort, but that reduces the seasonal coverage of samplings, would be the limiting of surveys to the richest season, such as spring. In the case of this study, spring sampling identified this season’s fauna quite well, as indicated by the low range of variation of all estimators used, and the high degree of similarity between observed and predicted richness scores. However, these estimators do not allow the further extrapolation of the sampled universe. Thus, although a reliable figure for the entire spring fauna can be obtained, the year-round species richness remains unknown.

Monthly estimates seem to be dependent on species richness. Predicted and observed species richness differs slightly from one to another of the richest months; this difference is greater in the species-poor summer months. Short-term spring sampling in April or May-June, with only 20 sampling effort units, seems to yield quite good estimations of the observed spring species richness and even of the predicted reasonably true spring species richness. The Jack2 estimator performed well in these extrapolations. Jackknife estimators in general, and Jack2 in particular, have been found to perform quite well, with greater precision, less bias and lesser dependence on sample size than other estimators, by many authors (Palmer, 1990, 1991; Baltanás, 1992; Brose et al., 2003; Petersen et al., 2003; Chiarucci et al., 2003). Accordingly, the use of this estimator is herein suggested to predict spring species richness, after previous assessment of inventory completeness by visual examination of the accumulation curve and a screening of the estimation deviations for the high levels of incompleteness which could alter estimation results. However, if inventories are less complete than the one analyzed herein, other orders of Jackknife estimators

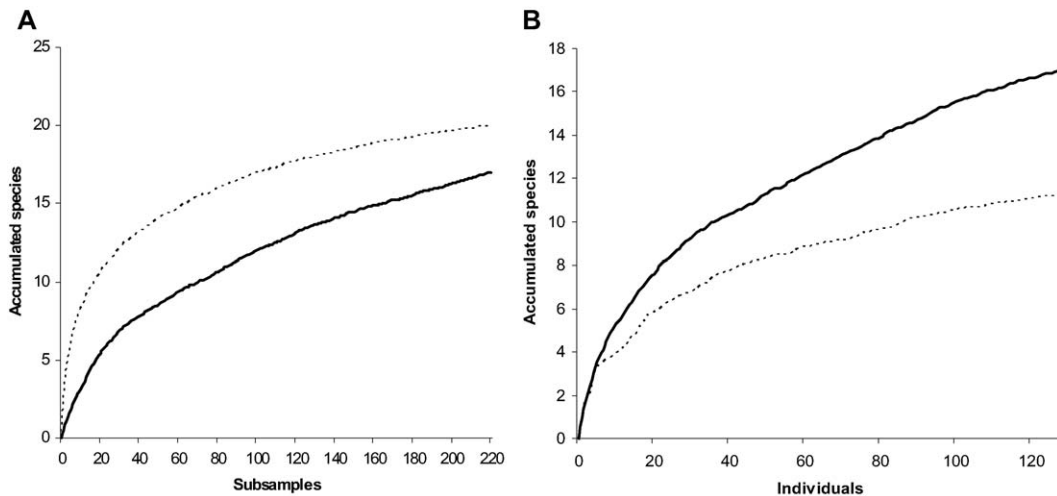


Fig. 7 – Randomized (500 times) subsample-based (A) and individual-based (B) accumulation curves for the annual inventory (solid line: juveniles excluded; dotted line: juveniles included).

should be used (Brose et al., 2003 for a detailed protocol to select estimator algorithms).

Thus, to optimize the field work involved in sampling the spider fauna (Araneidae and Thomisidae) of multiple sites under Mediterranean conditions, an exhaustive sampling protocol in one spring month is herein proposed. This strategy yields reasonable estimates of the entire spring fauna and, if spatial homogeneity of this observed pattern is preserved sites can be effectively compared, since spring inventories are a good representation both of the annual species richness and faunistic composition. Bearing in mind that a trade-off exists between survey effort and data quality, year-round sampling would be preferable if cost permits. Otherwise, optimal or spring samplings are two viable options; the former to be recommended if purely faunistic and taxonomic information (list of species) is of interest, the latter if a realistic picture of the yearly compositional structure is. If resources are extremely scarce, spring monthly sampling would be the preferred option. Although it is probable that a similar pattern may be common in many spider families, our conclusions must be restricted to Araneidae and Thomisidae because phenological patterns can vary among spider families.

The proportion of immature stages collected in this study, as in other spider studies, is very large (Coddington et al., 1996; Kuntner and Baxter, 1997; Cardoso et al., 2004; Sørensen, 2004). Because of demographic unavoidable reasons, rare species are more probably to be collected in early stages of the survey when dealing with juveniles, yielding a better representation of the complete assemblage more quickly (Fig. 7A). As the year-round sampling protocol, the effect of include juveniles diminishes the proportion of singletons and increases the asymptotic tendency of the accumulation curve. Moreover, the general shape of both accumulation curves, with and without juveniles, as well as their concomitant changes in steepness are different, because they constitute two different sampling universes (*sensu* Colwell and Coddington, 1994), altering estimates of phenomenological accumulation models (Fagan and Kareiva, 1997; Melo et al., 2003). The value of Weibull's asymptote, a function that fits data quite well (low sum-of-squares and high explained variance; see Flather, 1996 and Jiménez-Valverde et al., *in press*), is strongly influenced by the shape of the accumulation curve. Strikingly high estimates are derived from Weibull values obtained from curves whose slopes approach the asymptote more steeply towards their end, as occur in the adults-only accumulation curve. Overfitting is not a desirable property and the Clench model, less flexible and more conservative, approaches the asymptote with greater readiness than does the Weibull function yielding less biased estimations especially for non-asymptotic data sets.

Whatever the seasonal coverage of the sampling carried out adult-only specimen numbers greatly reduce the size of sample to be analyzed, and the faithfulness of sample representation of true species richness, while increasing the proportion of rare species (singletons, doubletons). All of the foregoing affects the performance of species richness estimators (Heltshe and Forrester, 1983; Smith and van Belle, 1984; Chao, 1987; Baltanás, 1992; Colwell and Coddington,

1994; Brose et al., 2003). Differences observed in the individual-based accumulation curves of adults-only and adults + juveniles data (Fig. 7B) are due to differences in the species-abundance relationships of both universes. In the adults + juveniles universe, common species have a higher relative abundance than in the adults-only universe. Thus, the higher observed species richness and more accurate estimations obtained when juveniles are included seem to be mainly due to the unavoidable increase in the number of individuals that thereby results. Definitely considering juveniles is necessary to obtain reliable estimates of species richness except when the sampling effort is so high that formerly unrepresented rare species emerge in the adult-only data set. Estimator robustness with respect to community structure changes is a desirable property (Melo et al., 2003) but, until now, no estimator has been shown to be so robust (Keating and Quinn, 1998; Baltanás, 1992). Accordingly, sample size must be large enough to include a high proportion of the true species richness and to represent the actual assemblage (Baltanás, 1992; Willott, 2001; Brose et al., 2003; Melo et al., 2003; Petersen and Meier, 2003; Petersen et al., 2003); and this can be better achieved including juveniles.

Identification of immature spiders is extremely difficult, especially in the tropics, where even many adult specimens must be classified as morphospecies because of insufficient taxonomic knowledge (Scharff et al., 2003). Even in temperate and Mediterranean areas, where the spider fauna is better known, identification of juveniles is not an easy task. However, in these geographic zones, surveys of a limited area for an extensive period provide reliable data on adult species composition and so could lead to the identification of many juveniles (e. g. Toft, 1976). Besides, ease of identification of immature stages varies with spider family and depends, mainly, on morphological distinctiveness and diversity of the taxa in the study area; e.g., juveniles of Gnaphosidae, Lycosidae or Linyphiidae are much more difficult to identify than those of the two families treated in this paper. When working with the entire spider fauna, using just identifiable juveniles will favor low diversity families versus high diversity ones, which probably contain the rarest species. This may introduce taxonomic and spatial bias when comparative studies are undertaken between families or sites, respectively. Much more research on juvenile identification, perhaps involving material from ecological studies, where large numbers of specimens are collected facilitating the match between immature and adult stages, should be carried out. As pointed out by Grove (2003), to maintain biodiversity project data integrity, juveniles and adults must be stored separately, in order to analyze them as advances in their identification are achieved.

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