

Seasonality of spiders (Araneae) in Mediterranean ecosystems and its implications in the optimum sampling period

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Abstract. 1. Fields such as ecology, macroecology, and conservation biology rely on accurate and comparable data. This is especially important for mostly unknown and megadiverse taxa such as spiders and regions such as the Mediterranean. Short-term sampling programmes are increasingly seen as the best option for sampling spiders. Comparability of results, however, demands standard procedures both in methodology and in sampling period. Cost-efficiency dictates that this period should be the most species rich.

2. Pitfall trapping was conducted in 23 sites from north to south Portugal, comprising three large-scale environmental zones and many different habitat types, during 10 months in each site. The annual richness pattern, differences in this pattern between areas and habitats, the complementarity between sampling periods and possible environmental correlates of richness were studied.

3. May and June present the optimal time for collecting spiders in Mediterranean areas. Northern areas have a later peak in richness and dense tree-cover sites offer more flexibility for sampling, with a higher proportion of species present at each period throughout the year.

4. Day length is the environmental factor most correlated with species richness. Maximum daily temperature may reduce richness, especially in southernmost areas, where summer temperatures can be extremely harsh.

5. It is recommended that short-term sampling programmes, intended to give a reasonable picture of spider communities in Portugal and in the Iberian Peninsula (and possibly extending to all the Mediterranean), should be conducted during May or June, with variable flexibility according to area and habitat. The proposed suggestions should appeal to everyone working in the field, given the cost-efficiency and comparability of results by adopting a common standardised approach.

Key words. Arachnids, biodiversity, complementarity, cost-efficiency, inventory, phenology, pitfall, Portugal, Spain, standardised protocols.

Introduction

Biological surveys inevitably produce samples of the total fauna, not complete inventories. If the databases used for (macro)ecological studies, or conservation priority analyses, are incomplete or inadequate, the result will often be inaccurate or

even misleading; however, incomplete data sets, if not exceedingly data deficient, can also provide good basis for different applications (Gaston & Rodrigues, 2003). Therefore, standardisation and calibration of sampling methods and protocols are fundamental for the comparison of sites or the definition of priority areas for conservation (Stork, 1994; Cannon, 1997; Jones & Eggleton, 2000; Kitching *et al.*, 2001).

Once one has chosen where to sample, there is the decision of how it is done. The sampling protocol and respective methodology are obviously taxon-specific. For spiders, complete protocols,

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or sampling packages, that gather information from a series of semiquantitative methods are considered the best approach for comprehensive and standardised sampling (Coddington *et al.*, 1991). They allow optimum results with a minimum effort, although this is always considerable. Such methods are suitable for capturing a large number of species and simultaneously a large number of specimens with replicate samples (Coddington *et al.*, 1996; Dobyns, 1997; Toti *et al.*, 2000; Sørensen *et al.*, 2002; Scharff *et al.*, 2003).

Besides deciding where and how to sample the question of when to sample must also be addressed. The answer relies fundamentally on the study of fluctuations in species richness of the target taxon according to the time of year, its phenology. Probably more important, it also relies on the degree to which such fluctuations are predictable year after year, its seasonality (as defined by Wolda, 1988). Highly seasonal phenomena can allow the reliable planning of fieldwork activities. Many studies are devoted to seasonal changes in abundance, the number of captured individuals (e.g. Chatzaki *et al.*, 1998, 2005a,b) or biomass (e.g. Pearson & Derr, 1986); however, the focus of this study is on species richness, not abundance or biomass, which mainly concerns individuals. Fluctuations in the richness of arthropods are usually much higher in temperate than in tropical regions, with the latter having a high proportion of species that are present throughout the year (Novotny & Basset, 1998). None the less, large seasonal differences in spider community composition, richness or abundance are invariably found, even surpassing habitat differences in many cases (Weeks & Holtzer, 2000; Whitmore *et al.*, 2002). Given these facts, one has to consider the implementation of long-lasting sampling programmes, of at least 1 year if not longer. Owing to the lack of human, material, and time resources, however, this is largely unfeasible. It is therefore imperative to optimise the duration of protocols and the time of the year when they are implemented. This is best achieved by carrying out standard minimum sampling programmes, which minimise the number and extension of collecting periods and simultaneously giving the most complete picture of diversity of any site (Oliver & Beattie, 1996; Duelli, 1997; Duelli *et al.*, 1999; Zonneveld *et al.*, 2003). All the protocols proposed to date have tried to answer the most obvious question: When should one collect to guarantee maximum diversity with minimum time spent on the task?

The first objective of this work was to choose the season that consistently presented highest richness in different geographical regions (corresponding to broad environmental zones as defined by Metzger *et al.*, 2005) and habitat types in Portugal. Second, to understand if and how do season, area, and habitat type potentially influence the sampled species richness. Third, to verify the effect on accumulated richness of increasing the number and duration of samples. Fourth, to assess if richness is correlated to any seasonal and easily predicted environmental factor. Finally, to give recommendations on the best sampling procedure for maximising spider species richness spending the least time possible in fieldwork, independently of area or habitat type in Portugal, the Iberian Peninsula, and possibly the Mediterranean area.

Materials and methods

Study sites

Four protected areas in Portugal were studied during three consecutive years, one year each area (Fig. 1): (i) Douro Internacional Nature Park (PNDI), located in north-eastern Portugal, sampled during 2001; (ii) Serras de Aire e Candeeiros Nature Park (PNSAC), located in central western Portugal, sampled during 2002; (iii) Paúl do Boquilobo Nature Reserve (RNPB), located very close to PNSAC, sampled during 2002; and (iv) Vale do Guadiana Nature Park (PNVG), located in south-eastern Portugal, sampled during 2003. These areas are located in the three environmental zones defined by Metzger *et al.* (2005) as being present in Portugal – Lusitanian (PNSAC, RNPB), Mediterranean North (PNDI) and Mediterranean South (PNVG). Only one of the zones present in the Mediterranean biome was not sampled, the aptly named Mediterranean Mountains.

In each protected area, most of its habitat types were sampled, summing 23 sites. A complete data set was therefore created, which allowed the comparison between many different habitat types in the different geographical areas and respective environmental zones (Table 1).

Sampling periods

Although the ideal situation would have been to sample all sites simultaneously, this was logistically unfeasible, given that the objective demanded to study areas as distant from each other as possible. Logistical reasons have also made it impossible to sample continuously during a whole year at each area. Given that December and January were thought a priori to be very poor in spider species, the remaining 10 sampled months were considered as representative of the entire year. All sites were sampled continuously, from the end of January (29–31, depending on the year) until the beginning of December (3–5). Sampling periods lasted for 2 weeks, providing 22 samples each year. Short-term sampling protocols usually take between 1 and 2 weeks of fieldwork and this 2-week period was adequate for the proposed objectives.

Sampling method

The main objective is to recommend the best time of year for collecting as many species as possible independently of the methods to be employed. To test all or even most of the available capture methods for several years and in so many sites, however, would be a formidable challenge. Therefore, a single method was chosen to meet the objectives, the one that could provide the most comprehensive information with the least effort. One of the most frequently employed and cost-effective methods for capturing spiders and other arthropods is pitfall trapping (e.g. Churchill, 1993; Samu & Lövei, 1995; Brennan *et al.*, 1999; Churchill & Arthur, 1999; Green, 1999; Norris, 1999; Riecken, 1999). Although pitfalls are not flawless (Adis, 1979) and exclusively directed towards epigeal species, they can capture almost half the

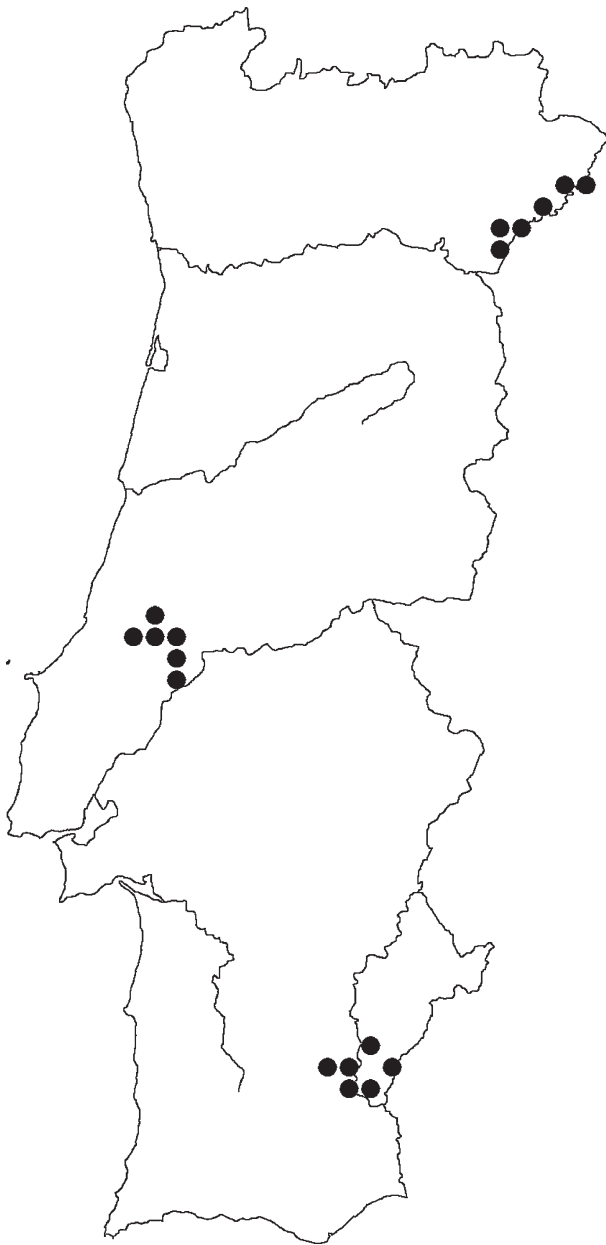


Fig. 1. Map of Portugal with all sampled sites according to Universal Transverse Mercator (UTM 10×10 km) squares. If several sites are located at the same square, only one symbol is represented.

spider species living in a typical Mediterranean habitat (Cardoso *et al.* unpublished data). Owing to differences in dispersal ability between arboreal and epigeal species, the latter constitute the bulk of Iberian and Mediterranean endemics and therefore the most important in conservation programmes, and the most dissimilar from site to site, allowing a better distinction between areas (Cardoso, unpublished data).

At each site, eight pitfall traps were set in a row, with 5 m separation between adjacent traps. Two plastic cups of 8 cm

mouth width, 12 cm depth and 33 cl capacity were used for each trap, one inside the other for easy emptying. Such cups were chosen over other available types because these are standard size plastic beer cups, easily available everywhere and rather inexpensive, two important issues to account for if a protocol is to be replicated by different teams. The preservative liquid was an antifreeze solution containing a proportion of ethylene glycol filling about two-thirds of the cup, on which some drops of detergent were added to break surface tension. Every trap was covered with a square wooden plate, about 2 cm above the ground. Only one of the sites, Paúl do Boquilobo (montado), underwent a greater sampling effort with eight rows of four traps each, to answer different questions that are outside the scope of this work.

Statistical analyses

Only adult specimens were considered for statistical purposes. Morphospecies were established whenever species could not be named. The designation 'species' is used throughout this work to name both identified species and unidentified morphospecies.

Hereafter, each fortnight sampling period will be referred as one sample. For all calculations, the percentage of species captured in each sample compared with the overall annual richness for each site was used as the measure of sample efficacy. This measure allows the direct comparison between all sites independently of their overall richness.

Usually, the higher the effort, the lower is the beta-diversity between units or samples (Colwell & Coddington, 1994) and sites with much different sampling effort could cause a systematic bias to results. Therefore, only one-quarter of the traps of Paúl do Boquilobo (montado) were considered, for standardisation of effort between sites.

Finding the optimum sampling period

To study the general pattern of diversity along the year different descriptors were considered, namely, the numbers of individuals and richness of families, genera, and species. Any sampling procedure, however, may introduce biases to data that are impossible to remove with statistical techniques. This includes species that are present at a site as active adults throughout a continuous season, but which are artificially fragmented in the data set by not being captured at every sampling period. Nevertheless, there are ways to mask such biases when dealing with large-scale phenomena, such as smoothing, which is frequently used to understand large-scale spatial patterns of species richness, given that data are often incomplete and blotchy (e.g. Prendergast & Eversham, 1997; Williams & Gaston, 1998). A smoothing technique much similar to the 'exponentially weighted central moving average' was used by considering the abundance value for each species per sample and site as:

$$n_s = 0.1n_{t-2} + 0.2n_{t-1} + 0.4n_t + 0.2n_{t+1} + 0.1n_{t+2}$$

where n_s is the smoothed abundance of species at time t , $n_{t \pm i}$ is observed abundance at time $t \pm i$ periods.

Table 1. Sampled sites with respective Universal Transverse Mercator (UTM) square (10 × 10 km), dominating vegetation and studied characteristics: location in the country (north, central, or south) and tree cover (dense, sparse, or open)

Site	UTM	Dominant vegetation	Location	Cover
Algodôr	29SPB07	<i>Quercus ilex</i>	S	S
Algozinho	29TQF07	<i>Fraxinus angustifolium</i> , <i>Salix salvifolius</i>	N	D
Bairro	29SND37	<i>Pinus pinaster</i>	C	D
Barrenta	29SND28	<i>Eucalyptus globulus</i>	C	D
Braciais	29SPB26	Resting wheat field	S	O
Bruçó	29TPF96	<i>Pseudotsuga menziesi</i>	N	D
Cerro das Antenas	29SPB16	<i>Pinus pinea</i>	S	D
Corredoura	29SPB17	<i>Quercus ilex</i> , <i>Juniperus turbinata</i>	S	S
Fonte d'Aldeia	29TQF18	<i>Quercus suber</i>	N	S
Limas (caminho)	29SPB28	<i>Cistus ladanifer</i>	S	O
Limas (ribeira)	29SPB28	<i>Nerium oleander</i> , <i>Phragmites australis</i>	S	S
Mazouco	29TPF85	<i>Cytisus scoparius</i>	N	O
Mértola	29SPB16	<i>Quercus suber</i>	S	S
Mira d'Aire	29SND27	Grassland	C	O
Palão	29TPF85	<i>Eucalyptus globulus</i>	N	S
Paúl do Boquilobo (montado)	29SND36	<i>Quercus suber</i>	C	S
Paúl do Boquilobo (salgueiral)	29SND35	<i>Salix</i> spp.	C	D
Picotino	29TPF86	<i>Pinus pinaster</i>	N	D
São Domingos	29SPB37	<i>Eucalyptus globulus</i>	S	S
Serro Ventoso	29SND17	<i>Quercus faginea</i>	C	D
Tó	29TQF07	<i>Quercus pyrenaica</i>	N	D
Vale Garcia	29SND37	<i>Quercus ilex</i>	C	O
Vila Chã da Braciosa	29TQF28	Resting wheat field	N	O

This technique is potentially useful because it provides a better picture of a community on a large-scale, but still it allows perceiving some small-scale phenomena. Other approaches have been tried to reduce bias in phenograms (e.g. Stamou & Sgardelis, 1989) but the approach here used is simpler when a large number of species is dealt with and equally efficient. For each of the five descriptors, both the proportion represented by each sample taking all sites into account and the average and standard error of this proportion for each site were considered. For subsequent calculations though, only the average proportion of species richness, which is the most commonly used measure of diversity and probably the simplest to understand, was considered.

Comparing areas and habitats

To study the effects of sampling period, geography (north to south areas and different environmental zones) and habitat type (namely tree cover density) in the proportion of species captured, all samples were classified according to their characteristics. Three-way ANOVA tests with those variables as independent factors were then performed to understand if groups significantly differed from each other for any of these factors. If so, an independent ANOVA test between each possible pair of characteristics allowed understanding which groups were different.

Optimising multiple samples

To study the effects of increasing the number and/or duration of samples, an iterative stepwise methodology applied to every site independently was used, allowing the application of the concept of complementarity, in this case, complementarity between sampling periods. Schematically, the approach can be represented in the following way.

- 1 Select the sample (period) that on average presents the higher proportion of species that occur at each site throughout the year.
- 2 Test if the selected sample is statistically different from the sample with lowest average proportion of represented species with an ANOVA. If not, stop here; it becomes statistically irrelevant which sample to choose next.
- 3 Remove from the data set all species already represented in the previously selected sample(s).
- 4 Recalculate the proportion of species represented in each remaining sample for every site in turn.
- 5 Return to step 1.

Testing possible environmental correlates of richness

To understand which, and how, environmental variables would help to predict the species richness of samples it is important to

know how this value changes with each available variable alone. The Spearman rank correlation with precipitation, minimum and maximum temperatures, and day length was calculated for the purpose. The average daily values of environmental factors across each sampling period were taken as independent variables, given that many times the available data was fragmented in time and some of the values were impossible to obtain. In addition, only meteorological data from 2002 and 2003 of a single site close to the collecting areas was available, Santarém (2002, central Portugal) and Vale de Camelos (2003, southern Portugal). Therefore, only these two years and areas were considered for analysis. Correlation values were calculated between the average richness of each sample and the selected abiotic data. If the correlation was found to be significant ($P < 0.05$) at least during one of the years, a linear regression line to the same data was fitted in order to visually understand how richness changes with the environmental variables. If the correlation was not significant for at least one of the years, a second-degree polynomial equation was fitted to the data. Finally, forward stepwise multiple regression analysis with all available variables was used to understand how these variables could potentially explain sample richness. All statistics were performed with Statistica 6.0 software package (Statsoft Inc., 2001).

Results

A total 11 275 adult spiders were collected, representing 36 families, 151 genera and 345 species (list available in Cardoso, 2004). Gnaphosidae is the most diverse family, with the highest overall species richness. The distribution of species along the annual cycle presents some noteworthy features, confirmed by the data and pers. obs. during fieldwork. Most families have maximum richness in May and June but a notable exception are Linyphiidae, which are most rich and abundant during winter months. Most of the larger and stouter species are only captured as adults in late summer and autumn [e.g. *Lycosa tarantula* (Linnaeus, 1758), *Hogna radiata* (Latreille, 1817), and *Argiope* spp.], even if adults live hiding during the rest of the year [e.g. all Mygalomorphae like *Atypus affinis* Eichwald, 1830, *Iberesia machadoi* Decae & Cardoso, 2005, *Nemesia* spp., and *Ummidia aedificatoria* (Westwood, 1840)]. Many species have a notoriously stenochronous pattern, with population outbreaks during very short time periods of less than 1 month, adults being entirely absent in captures during the rest of the year [e.g. *Alopecosa albofasciata* (Brullé, 1832), *Harpactea fageli* Brignoli 1980, *Ozyptila pauxilla* (Simon, 1870), and *Zodarion merlijni* Bosmans, 1994].

Optimum sampling period

As expected, the sampling period is the single factor that most strongly affects the percentage of species captured at any time in any site (Table 2). The distribution of abundance and richness in all tested forms generally follows a consistent pattern (Fig. 2). The peak is always reached during May but a high plateau is following throughout June. All graphs also present a second, more or less pronounced peak around October. Of the

first and largest peak, two samples have the higher proportion of species, the ones from mid-May to early June (samples 8 and 9 of 22 in Fig. 2d). Each of these two samples is enough to capture on average per site: 7% of the individuals, 45% of families, 29% of genera, 23% of species or 42% of species with smoothed data. Of the two, sample 9 (late May and early June) presents a much lower standard error of the percentage of species captured in each site in all cases, assuring with higher confidence that the highest percentage of species is captured if only one sample is to be made in any place.

Comparison between areas and habitats

Northern areas appear to have their richness peak 2 weeks later than the other regions (Fig. 3a). On the other hand, central areas and dense tree cover sites present an earlier raise in richness compared with the other sites (Fig. 3). Both area and habitat affect the proportion of species that are present during the year in any site (Table 2). None of the interactions significantly affects this value, though. The effect of geographical area (and respective environmental zone) is not easily explained given that no gradient is found, central Portugal (in the Lusitanian environmental zone) has a considerably higher overall percentage of species present throughout the year (Table 2, Fig. 3a). Northern and Southern Portugal are not possible to tell apart in this respect (Table 2). The tree cover density of a site seems to affect species richness in a clear way, with dense tree cover sites having a higher percentage during most of the year and open to sparse tree cover habitats presenting no differences between them (Table 2, Fig. 3b).

Multiple samples

Beginning at sample 9, the one previously chosen as corresponding to the best time for a single sampling period, the iterative method was applied. The next three samples being selected were all between mid-April and very early July (samples 6–11;

Table 2. Three-way ANOVA and paired ANOVA results for areas and habitats

Independent variable	d.f.	F	P
Area	2	6.237	0.002
Habitat	2	5.120	0.006
Sampling period	21	13.690	<0.001
Area × habitat	4	1.064	0.374
Area × sampling period	42	1.303	0.109
Habitat × sampling period	42	0.586	0.981
Area × habitat × sampling period	84	0.941	0.623
Area (geographical region)			
South × centre	1	18.740	<0.001
Centre × north	1	10.460	<0.001
South × north	1	1.366	0.243
Habitat (tree cover)			
Open × sparse	1	0.073	0.787
Sparse × dense	1	10.350	<0.001
Open × dense	1	9.598	0.002

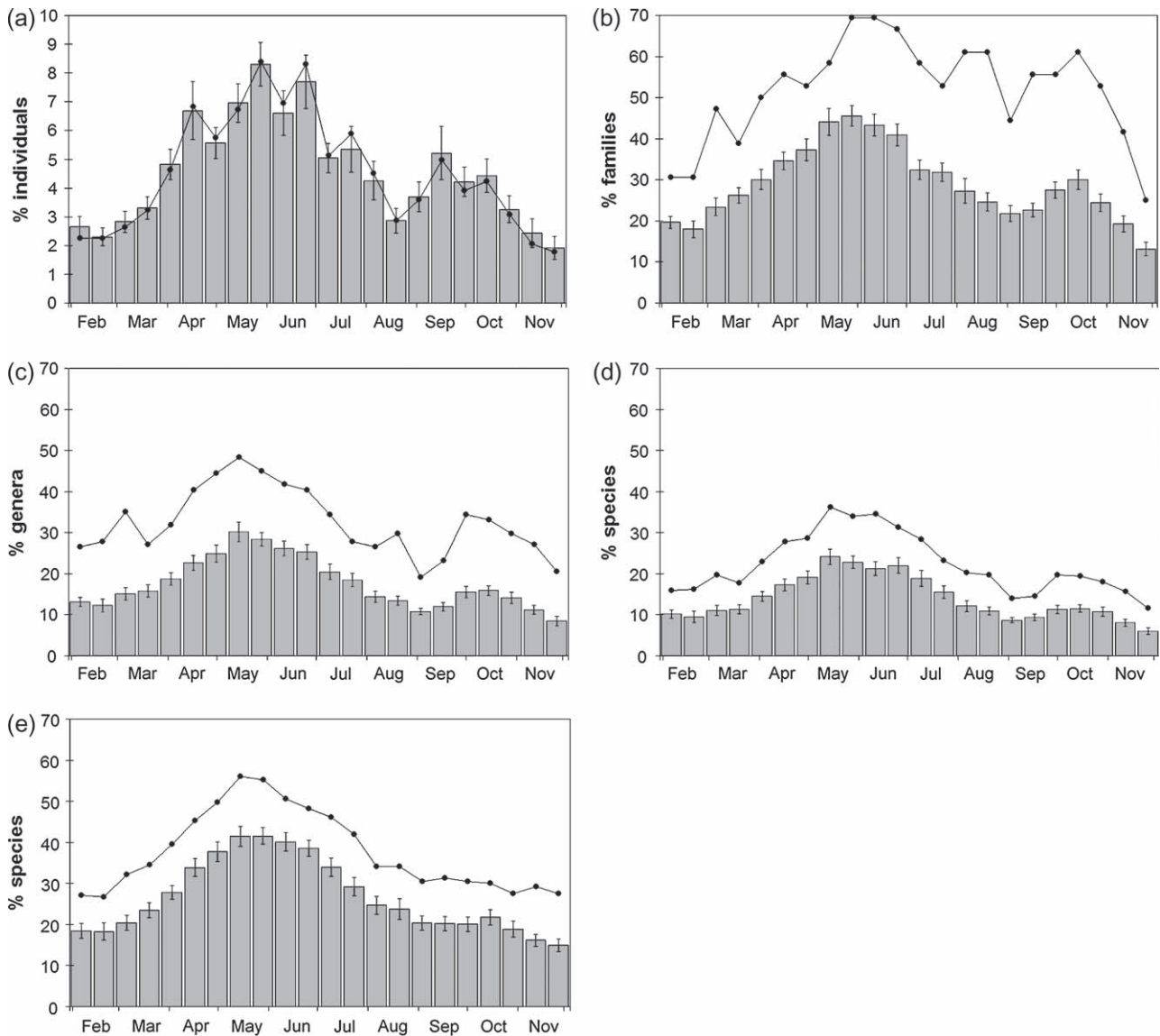


Fig. 2. Average percentage per site (bars) and total percentage including all sites (line) of abundance or richness considering: (a) individuals; (b) families; (c) genera; (d) species; and (e) species with smoothed abundance values (see text for explanation of smoothing procedure). Error bars represent the standard error.

Fig. 4). Only after these, a sample belonging to a different season, coincident with the autumn hotspot, was chosen (sample 19, during mid-October). After it, new samples from the same season as before present more species not represented already. Although sample 7 was the next being chosen by the method, the difference in average species richness to the lowest richness sample is no longer statistically significant ($P > 0.05$), becoming irrelevant which sample to add after sample 10.

Environmental correlates of richness

High temperatures with practically no precipitation during summer, and mild, rainy winters characterise the Mediterranean

climate. Temperature maximum and annual amplitude can be very high in interior areas, such as is the case of PNVG, but are relatively low in coastal areas, such as in PNSAC and RNPB. Precipitation, on the contrary, is usually higher in coastal and northern areas (Fig. 5a). Precipitation was found to be negatively correlated with richness during 2002 ($r_s = -0.624$; $P = 0.002$) and marginally correlated during 2003 ($r_s = -0.419$; $P = 0.052$). Temperature was not found to be linearly correlated with richness in any case (Fig. 5b,c; in all cases $r_s < 0.382$; $P > 0.152$). All the polynomial curves indicate a humped shape of the relation, with mild temperatures allowing maximum richness and the typical high summer temperatures causing a depression on the number of species that are active. By far, day length was the environmental variable most correlated with

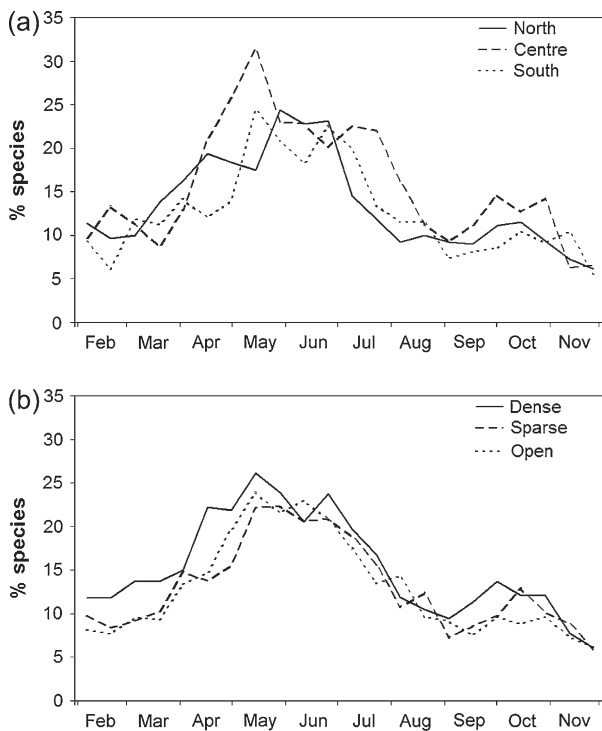


Fig. 3. Average percentage of species with sites divided according to: (a) geographical location in Portugal, and (b) tree cover density.

richness (Fig. 5d; in both cases $r_s > 0.756$; $P < 0.001$). As already pointed out, the differences in explanatory power and curve shape of environmental variables can be caused by the high-summer temperatures, which is confirmed by the multiple regression results (Table 3). In both models, day length is the first variable to enter, in fact the only variable in the 2002 case; however, in the 2003 model maximum temperature is also included, with a negative regression coefficient. Given that 2003 sampling was made in the warmest part of the country,

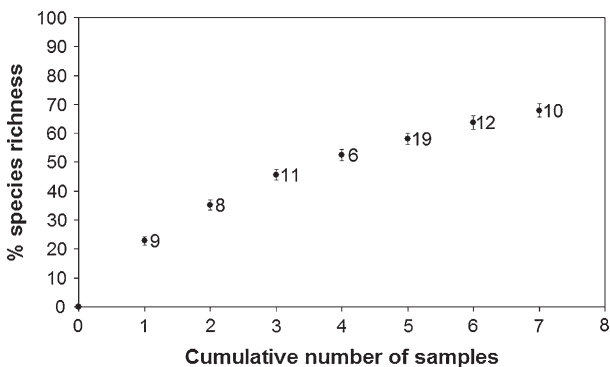


Fig. 4. Cumulative percentage of species richness possible to capture by adding samples, according to the ones that, at each step, further increase the average number of species represented. Added samples at each step are indicated next to the respective symbol. Error bars represent the standard error. Beyond seven samples, it becomes statistically insignificant which sample to add at the next step.

maximum temperature did have a strong depressing effect on the activity of most species, which did not happen during the previous year.

Discussion

In one of the few published studies on ground spider seasonal richness variation in the Mediterranean (Chatzaki *et al.*, 1998), conducted in the eastern Mediterranean, the dominant family captured by pitfall traps was, as in this case, Gnaphosidae. Apparently, this is a feature common to all Mediterranean biomes. Coincidentally, this family was also found to be the best surrogate for predicting overall Araneae richness in Portugal (Cardoso *et al.*, 2004b). The same authors have also found the discrepancy of Linyphiidae abundance during the yearly cycle compared with most other families. This family as a whole is the most abundant and species rich in temperate ecosystems of the northern hemisphere at all times, but only during winter in Mediterranean areas.

Chatzaki (pers. comm.) found the same annual richness pattern for Gnaphosidae in Crete, and Jiménez-Valverde and Lobo (2006) for Araneidae and Thomisidae in central Spain, as we have found for Araneae in Portugal. This pattern is different from the one usually found in higher latitude where summer months frequently present a richness peak (e.g. Duffey, 1962; Merrett & Snazell, 1983; Niemela *et al.*, 1994). The same pattern occurs with other arthropod groups such as butterflies in high-altitude habitats of the Iberian Peninsula (Gutiérrez & Menéndez, 1998). Summer drought is only mitigated with an increase in either latitude or altitude.

All tested richness and abundance values agree on the general shape of the curve, only differing on the sharpness of richness peaks, and mostly on the proportion of taxa that can be found on average in each sample. The latter difference is expected, given that as happens with higher taxa surrogacy cases, the higher the taxa, the faster its richness reaches high completeness levels, with the continuous adding of individuals or samples (Cardoso *et al.*, 2004a).

Most remarkable are the differences between using raw or smoothed data of species for calculating richness. Both the smoothed data and the iterative approach reach $\approx 50\%$ of overall annual species richness during the May/June samples, suggesting that this percentage is probably close to the real maximum proportion of species that coexist as active adults at any one time in a single site from the annual, total pool of species. The remaining species should be present at the site, but in egg or juvenile stages or simply inactive. Other authors in the Iberian Peninsula (Jiménez-Valverde & Lobo, 2006) and the rest of Europe (e.g. Scharff *et al.*, 2003) reached this same proportion at the same time of year. This is a sign that the smoothed values are probably more precise in the percentage of species represented during each season. Nevertheless, smoothing gives a less precise perception of small annual fluctuations of richness, masking the second richness peak.

From the samples that were found consistently presenting the higher proportion of species, the one from end of May and beginning of June (sample 9) gives more confidence for future

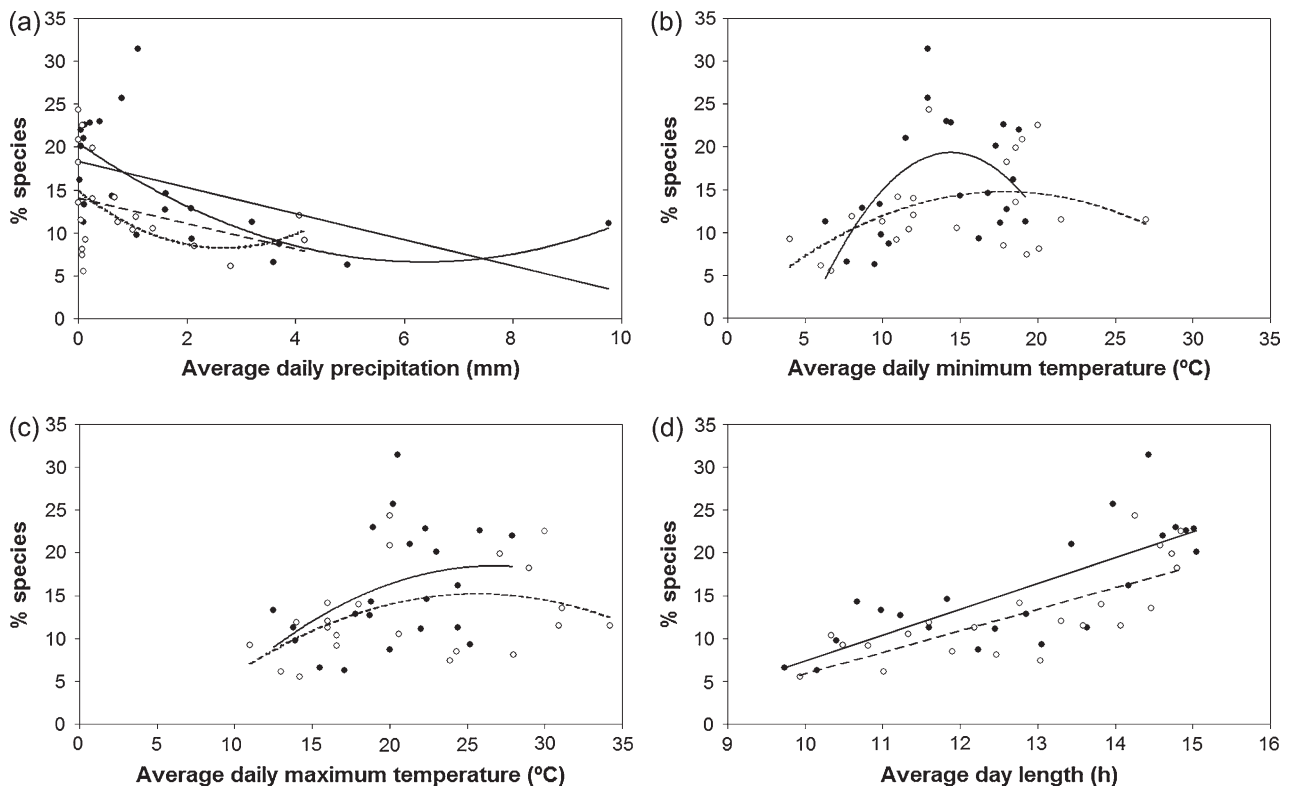


Fig. 5. Species richness values related to average values of climatic variables during each sampling period: (a) precipitation; (b) minimum temperature; (c) maximum temperature; and (d) day length. Either linear or second-degree polynomial functions were fitted according to which would allow a better visual assessment of the relation between richness and independent variables. Solid line and black dots represent the 2002 data, dashed line and open circles represent the 2003 data.

sampling. Its standard error is lower and it is closer to the middle of the high richness season. Besides, northern sites present a later peak in richness than the rest of the country, mostly coinciding with this sample.

The richness values of the sites in the centre of the country and with dense tree cover rise sooner and are generally higher throughout the year. A dense tree cover causes smaller meteorological variations, creating a more stable microclimate below the canopy throughout both daily and yearly cycles. Central Portugal sampling was made in the Lusitanian environmental zone, which is characterised by lower summer temperatures, higher precipitation and a relatively mild climate compared with

the typical Mediterranean areas. In addition, more sites with dense tree cover were sampled in these areas, possibly confounding the effects. Both causes explain that this central area follows the same pattern as dense tree cover sites. Moreover, stochastic effects make each sampling year different even in the same sites, which means that analysing the habitat data is more robust given that habitat types were distributed among all areas and years.

Complementarity is inherent to the iterative method used in the accumulation of samples, so the ones to be chosen after the first sample were expected to present a large time lapse in between. Up to the fourth iteration though, all samples belong to the spring season. This can be caused by the low effort employed, which artificially increases complementarity values between samples that should be very similar (Colwell & Coddington, 1994; Cardoso & Borges, submitted). Nevertheless, it unquestionably brings up one conclusion: to maximise species richness, it is better to make a larger effort in May and/or June before considering spreading the effort to other periods. This approach adds the advantage that cost is usually much lower and resources simpler to guarantee during short periods.

Many studies have been published trying to explain seasonal patterns of species richness, in all kinds of biomes, most of the time considering environmental factors. Precipitation and temperature are probably the two most tested variables and in fact

Table 3. Forward stepwise multiple regression analyses for each year (species richness as dependent variable). 2002 in central Portugal (final model): multiple $r^2 = 0.595$, $F_{3,18} = 13.261$, $P < 0.001$. 2003 in southern Portugal (final model): multiple $r^2 = 0.481$, $F_{2,19} = 20.606$, $P < 0.001$

Year	Environmental variables				
	entering final model	β	SE of β	t	P
2002	Day length	0.915	0.207	4.4	<0.001
2003	Day length	1.061	0.179	5.9	<0.001
	Maximum temperature	-0.420	0.179	-2.3	0.030

β is the standardised regression coefficient, t represents the test statistic.

can be the most important factors leading to changes in abundance and richness of arthropods in sub(tropical) areas, especially the first (Wolda, 1978, 1988; Basset, 1991; Pinheiro *et al.*, 2002). In such areas, the wet season usually reveals more species, individuals, and biomass of arthropods (Wolda, 1978, 1988; Pearson & Derr, 1986; Basset, 1991; Pinheiro *et al.*, 2002). On the contrary, temperate and Mediterranean species are usually intolerant to rainfall (Fig. 5a). As predators, spider richness is ultimately driven by the existence of prey (mainly insects) and respective food sources (mainly new leaf production), which are especially abundant during the rainy season in tropical forests, but only after the end of winter in Mediterranean areas (e.g. Radea, 1993). In temperate climates, many taxa peak during summer. This can be due to a long winter, which narrows the possibilities of species to develop, but even purely geometric constraints taking no variability of abiotic factors into account were already proposed to explain this mid-season richness peak (the mid-domain effect; Morales *et al.*, 2005). Almost all of the studies though recognise the importance of temperature variability, either in explaining the pattern or the shifts from it. In this case, maximum temperature in fact explains the summer depression in richness, especially determinant in the warmer parts, corresponding to the 'Mediterranean South' environmental zone, although it does not explain the general pattern, nor does precipitation. The factor most strongly correlated with richness is day length. This variable was seldom, if ever, recorded in literature as a driving factor of species richness. It is in fact hard to explain why the correlation is so high and most probably there is no causal relation. Many species of spiders have nocturnal activity in order to escape the high diurnal predatory pressure and winter and summer day length is not extremely different at these latitudes (9–15 h respectively). Nevertheless, the purpose of this work was not to establish a causal relation, but to know if the richness peak is predictable independently of year, area, and habitat. Given that a sampling season is almost invariably planned well in advance, one must do it according to a time lapse that allows to predictably capture many species with a certain degree of confidence, independently of weather. Moreover, for comparison between sites sampled in different years it is far easier to justify their comparability if the sampling period remained constant, instead

of relying on environmental variables. Such measures may even be unavailable in many cases and it is often logistically unfeasible to plan a sampling period only a few days in advance (but see Duelli *et al.*, 1999). Finally, given that the data suggest day length as the main factor correlating with species richness, the best collecting period is highly predictable anywhere in the study area.

Considering all possibilities and limitations, the end of May to early June is most likely the best time for investing in collecting spiders in Mediterranean habitats if the objective is to do short term intensive sampling (Table 4). The beginning of and mid-May can also be thought of as an optimum time but preferentially not in northern regions, where there is a chance that the hotspot has still not occurred. The sampling season may also extend throughout June, except in southern regions, where high maximum temperatures may cause a drought in richness. If, for logistic or any other reasons, sampling is done outside the proposed periods, it should be only in dense tree cover sites, where a larger flexibility in the optimum sampling season is to be expected.

Some cautionary remarks must be made. This study was restricted to ground spiders. Given the three-dimensional nature of most habitats the reached conclusions may not be optimum for arboreal spiders. Moreover, more extreme environments can be present in the Mediterranean area and sampling in such sites may require a shift to slightly different seasons. Lastly, these conclusions apply to studies of overall species richness. If the objective is to study one particular family (e.g. Nemesiidae or Linyphiidae), however, the optimum sampling period may be very different.

Nevertheless, given the wide variety of habitats and areas now studied, comprising most of the environmental zones present in the Mediterranean region, the conclusions now presented should apply to the entire Iberian Peninsula and possibly the Mediterranean region. Following the suggestions here presented will allow two major advantages. First, by sampling during the richest period, maximum cost-effectiveness of the protocol to be applied is guaranteed. Second, the congruence in sampling period will necessarily be reflected in the increased congruence of comparisons between different studies. The next logical step will be to test in Mediterranean habitats, and propose for large-scale use, standardised and optimised sampling protocols to be applied during these periods.

Table 4. Adequacy of periods for sampling according to geographical area in Portugal and habitat (tree cover density) characteristics

		April		May		June		July	
		1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half
North	Dense	–	–	o	+	+	+	o	–
	Sparse	–	–	–	o	+	o	–	–
	Open	–	–	–	o	+	o	–	–
Centre	Dense	–	o	+	+	+	+	o	–
	Sparse	–	–	o	+	+	o	–	–
	Open	–	–	o	+	+	o	–	–
South	Dense	–	o	+	+	+	o	–	–
	Sparse	–	–	o	+	o	–	–	–
	Open	–	–	o	+	o	–	–	–

–, avoid; o, good; +, optimum.

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