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Determining spider (Araneae) species richness in fragmented coastal dune habitats by extrapolation and estimation

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Abstract
In this contribution we review which species richness estimators can be used if spiders are sampled with pitfall traps or other relative sampling methods. Due to the inherent bias typical for activity-based traps, only sample-based estimators were useful (Chao2, ICE and two jackknifing estimates). We estimated species richness for spiders from four fragmented coastal dune habitats: dense and short grasslands, moss dunes and marram dunes. As extrapolation of the collector curve is only appropriate if the curve reaches a ceiling (asymptotic, such as parabolic and hyperbolic models), total species richness could not be determined by extrapolation in either of the investigated habitats. Because of the log-linear nature of the curves, non-parametric methods were applied. An absolute estimate of total habitat species richness was thus difficult to calculate; differences in total regional species richness could be analysed by comparing the different non-parametric estimator curves. If only habitat specific species were taken into account (we analysed this for grey dune habitats), extrapolation of the collector curve was appropriate (hyperbolic models). A relatively low number of traps were already enough for sampling more than 95% of the specific species. Estimates of the richness of specific species from grey dune patches (in contrast to species richness of the habitat) could already be derived from five traps. Our data revealed that grassland habitats were characterised by higher spider richness than moss-dominated grey dunes and that the latter were more diverse than Marram dunes. The number of specific species was slightly, although non-significantly, larger in dune grasslands than in moss-dominated dunes.

Key words: estimators, xerotherm species, grey dune, Marram dune, dune grassland

INTRODUCTION

The quantification of species richness for terrestrial arthropods is important for habitat conservation studies (May 1988). Pitfall sampling is a time- and cost-effective collecting technique (Sunderland et al. 1995). However, a trade-off exists between sampling energy and sampling precision since pitfalls register species activities and not only species abundances. Parameters determining the sampling success are species densities, activities, stage and species-specific trappability (Sunderland et al. 1995). Additionally, the efficiency of pitfall traps is also strongly influenced by the surrounding vegetation (Sunderland et al. 1995). The estimation of arthropod species richness thus strongly depends on the probability that all species can be sampled. Since cursorial activities are species-specific (Bonte et al. 2000), dependent on vegetation structure and even on the population and prey density (Kreiter & Wise 2001; Bonte & Maelfait unpub. data), estimates of species richness based on the relative densities will be heavily biased and incomparable between different and even similar arthropod assemblages.

Colwell & Coddington (1994) give an overview of methods for estimating local species richness by extrapolating species accumulation curves, fitting parametric distributions of relative abundance or using non-parametric techniques based on the distribution of individuals among species or of species among samples. They also stress the need for the evaluation of these estimators with real data for a diverse range of organisms and habitats. In this contribution we investigate, based on a small amount of pitfall traps, which species richness estimate should be used for the assessment of spider species richness in some fragmented coastal dune habitats.

MATERIAL AND METHODS

Data collection

For the estimation of species richness based on pitfall trapping, we analysed data on spider (Araneae) presence from a large pitfall sampling campaign in the Flemish coastal dunes, in which data from more than 178 year-round pitfall captures were included (Bonte et al. 2002). In each sampling station, three to five traps were regularly placed, with a distance of 5-10 m between each pitfall (the traps were glass jars with a diameter of 9.5 cm, filled with 10% formaline solution and fortnightly emptied). All dune habitat types were sampled from 1975 onwards and in total more than 65000 adult spiders were identified, resulting in data on the presence of 214 species.

In this contribution, we analyse data from four intensively sampled habitat types that are heavily fragmented due to shrub encroachment (Provoost et al. 2002): Marram dunes (blond dunes near the seaside, dominated by Marram grass *Ammophila arenaria*), moss-dominated dunes, short dune grasslands and dense grasslands. The numbers of traps (samples), placed in each of these habitats are listed in Table 1. For these habitats, we tried to estimate the regional habitat species richness, i.e. the total expected number of species of the particular habitat from the coastal dune region of Flanders (Belgium). Specific species (stenotopic xerotherm species, typical for either short grasslands and moss-dominated dune habitats in our region, but also occurring in other habitats like heathland and chalk grassland (based on Hänggi et al. 1995; Maelfait et al. 1998; Bonte et al. 2002, all listed on the Red List of the spiders from Flanders – Maelfait et al. 1998; see Appendix for an overview)) were also selected from this data set to estimate the total number of specific species in the four habitats. Especially the estimation of the total number of specific species is important in bio-indication (Bruun 2000; Bonte et al. in press)

Preparation of the collector curves

A ' collector curve' or ' species accumulation curve' is a plot of the cumulative number of species *S* discovered within a defined area, as a function of some measure *n* of the effort to find them (Colwell & Coddington 1994). The most straightforward measure of effort is simply the number of individuals or samples. Because of the bias in individual numbers, we used the number of samples as measure of effort (Gotelli & Colwell 2001). In collector curves, the order in which samples are added to the total affects the shape of the curve. To eliminate this arbitrariness, sample order is randomised 1000 times (or less in case of $n<7$) and the mean and standard deviation of $S(n)$ are computed for each value between 1 and *n*. Collector curves were computed with *EstimateS* (Colwell 2000).

Fitting models to the collector curve

The collector curves for the regional habitat species richness were fitted with three empirical mathematical models in order to describe the nature of these curves (Colwell & Coddington 1994; Samu & Lövei 1995; Gotelli & Colwell 2001). We computed an asymptotic, negative exponential model, based on the equation $S(n)=S_{max}\ (1-e^{-Kn})$; and an asymptotic two-parameter hyperbola model, $S(n)$ =

$(S_{max}n)/(B+n)$, where $S(n)$ is the number of species in the n[th] cumulative sample, S_{max} is the estimated true richness, K and B estimated constants for the site. A non-asymptotic log-linear model was also fitted where $S(n)$ was assumed to be a linear function of the logarithm of the sample number n. The amounts of variance explained by the different models were compared to find the best fitting model. If the asymptotic models gave a better fit than the non-asymptotic one, then no estimates were used since total richness could be derived from the asymptote (Colwell & Coddington 1994). In the case of a non-asymptotic fit, four species richness estimator curves were constructed. The computation of these estimator curves proceeded in the same way as that of the collector curves. Instead of the number of observed species S for a given sample size n, however, the estimate for this sample size was calculated, randomised and averaged. Because of the randomisation procedure, standard deviations could again be calculated. These were fitted against the asymptotic and non-asymptotic models for evaluating their behaviour. The assumption that the number of singletons should reach a peak at about half of the sampling effort was fulfilled for the regional habitat species richness estimating procedure, but not for the estimation of the total patch species richness, because these were based on a low number of traps (Bonte et al. unpub. data). Because of the low number of traps in each patch, the collector curve for five traps was reconstructed with data from 12 patches of moss dominated dunes and short grassland. The nature of this curve was visually interpreted since model fitting with such a small amount of samples seemed to be inappropriate.

The use of appropriate species richness estimators

In Table 2 we give an overview of the appropriate species richness estimators that can be used. We regarded individual-based estimating methods (ACE, Chao 1) and the use of species richness indices to be inappropriate, because of considerable species-specific variation in capture probabilities and species specific trapping trappabilities (Sunderland et al. 1995). As a result, methods that rely on relative densities (Colwell & Coddington 2000) make comparison a priori impossible. Hence, we estimated species richness by computing collector curves, as mentioned before and by constructing estimator curves for the four non-parametric estimates: ICE, Chao2 and two jackknife methods, which are based on the number of rarely (species only found in one – singletons - and two - doubletons - samples) captured species (Colwell & Coddington 1994; Colwell 2000). All analyses were done with the software package *EstimateS* (Colwell 2000).

RESULTS

Regional habitat species richness

Shape of the species collector curve

The collector curves did not reach an asymptote for either of the included species in the

Table 1. Total number pitfall traps used to estimate species richness in the four sampled habitats and for richness in specific species for moss dune and short dune graslands. R^2-values for the three types of fitted curves on the species collector curve are given. In bold: best fitting curve.

Habitat	n	$R^2_{Hyperbola}$	$R^2_{Assympt}$	$R^2_{log\text{-}lin}$
Marram dune	25	0.993	0.935	**0.996**
Moss dune	26	0.993	0.954	**0.996**
Short grassland	41	0.987	0.937	**0.999**
Dense grassland	52	0.977	0.947	**0.999**
Moss dune specific species	26	**0.998**	0.975	0.949
Short grassland specific species	41	**0.999**	0.978	0.985

Table 2. Overview of the most commonly used species richness estimators with an indication of what they are based on, and their suitability for species richness estimation in arthropod (pitfall) sampling.

Estimator	based on	suitability
Species richness indices	individuals	absolute sampling techniques
Extrapolating collector curve	individuals	absolute sampling techniques
Extrapolating collector curve	samples	pitfalls if asymptotic collector curve
Rarefaction	individuals	absolute sampling (interpolation, for detecting dominance effects)
Chao 1	individuals (rare species - occurring in one and two individuals)	absolute sampling
Chao 2	samples (species occurring in only one and two samples)	pitfall trapping and other relative sampling techniques
ACE	individuals	absolute sampling techniques
ICE	samples	pitfall trapping and other relative sampling techniques
Jackknife 1	samples (species occurring in only one sample)	pitfall trapping and other relative sampling techniques
Jackknife 2	samples (species occurring in only one and two samples)	pitfall trapping and other relative sampling techniques

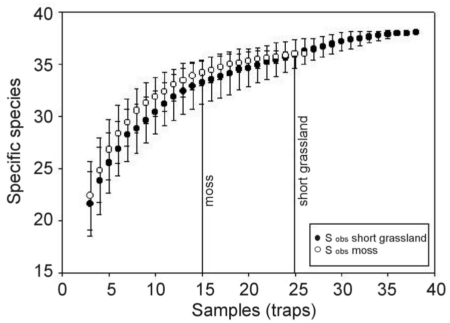


Fig. 1. Collector curves for specific species from two types of grey dunes in the Flemish coastal dunes, with indication of the minimal amount of traps to sample 95% of the species (vertical lines). Mean values of the oberved number of species and standard deviations are given.

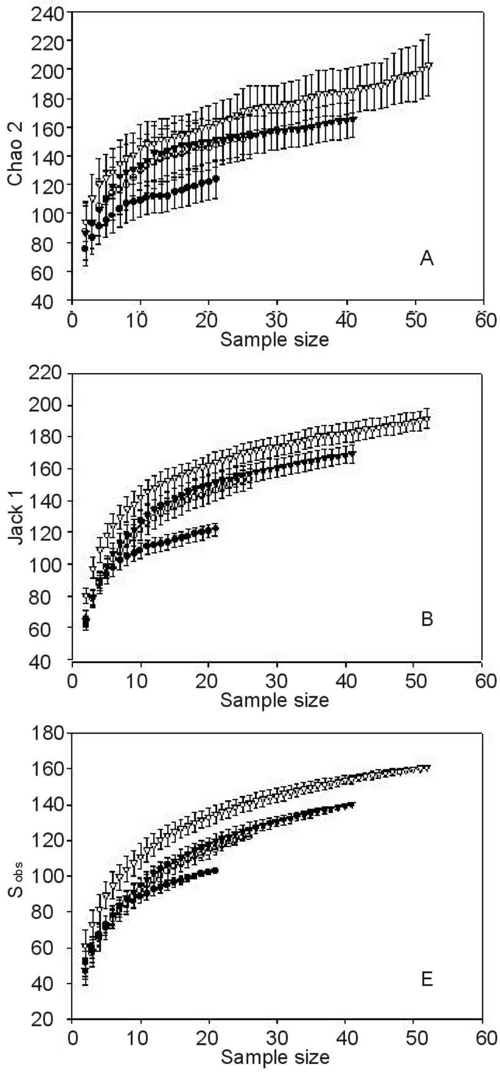
four investigated habitat types, since neither the hyperbolic nor the asymptotical curve fitted better than the log-linear one (Table 1).

The collector curves for the specific species for grey dunes (moss dunes and short grasslands) were asymptotic, indicating that all specific species were caught with the total number of applied traps (Table 1). As a consequence, the asymptotic value gives a good reflection of the total number of specific species (Fig. 1): 36 species in the moss dominated dune and 38 in the grassland. Standard deviations on the estimate for the two habitats overlap, so differences are not significant. In moss dunes, more than 95% of all the specific species in the region could be captured with 15 traps; in dune grasslands, 25 traps were necessary (Fig. 1).

Estimating and comparing regional habitat species richness

The shape of the estimator curves are visualised in Fig. 2. None of the curves could be fitted better by an asymptotic than by a log-linear regression, as revealed by comparing R^2-values of the asymptotic function and the log-linear for each estimator in each habitat (mean $R^2_{asymptotic}$=0.872; mean R^2_{loglin}=0.978 ; paired t_{15}=-4.41; P<0.001). This means that the estimate did not reach an asymptote, but increased with sampling size. Since regression slopes were also different, an estimate based on a fixed number of samples could neither be applied for estimating the hypothetical total number of species, nor for comparing species richness between habitats.

In contrast, we could study differences in estimated regional habitat species richness by comparing the behaviour of the estimate- and collector curves (Fig. 2). According to the Chao2 curve we can conclude that $S_{marram\ dunes}<S_{dense\ grassland}$. All other estimators indicate additional differences: $S_{marram\ dunes}<S_{moss\ dune}=S_{short\ grassland}<S_{dense\ grassland}$. So, although no exact estimation of total species richness can be calculated, the sequence in regional habitat species richness is clear.



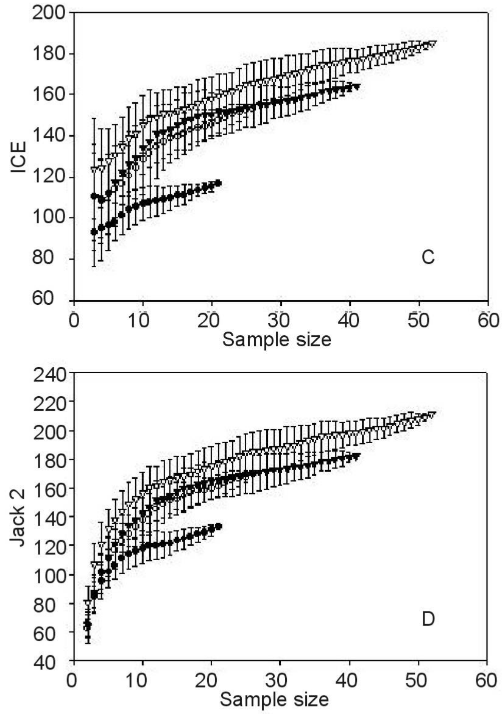


Fig. 2A-E. Performance of the four non-parametric estimators of species richness (**A**) Chao 2; (**B**) ICE; (**C**) Jackknife 1; (**D**) Jackknife 2, and the collector curve (**E**) Sobs for the empirical data set from the four different types of dune habitats. Mean values of the estimated number of species and standard deviation are given.

Patch species richness

If patch species richness, as opposed to regional habitat species richness of the two grey dune habitats (short grassland and moss dunes) is to be investigated, the same procedure as above can be applied. Since the number of singletons increased linearly with the increasing number of traps (linear regression, R^2>0.90 for all patches), no estimates could be calculated for patch species richness, because of the low number of traps. If only xerotherm dune specific species were taken into account, the number of singletons was typically the highest for three traps and declined for four and five traps (Bonte et al, unpub. data). However, the collector curve (visually) reached a ceiling from three samples onwards and did not increase linearly anymore (Fig. 3). Estimating patch species richness was thus impossible for all species, but proved to be realistic for the number of observed specific species. Only very rare specific (endogeic) species like *Acarthauchenius scurillis* and *Mastigusa arietina* were rarely captured with a low number of traps.

Total patch species richness can best be compared using the total number of observed species or a richness estimate, based on the same amount of traps (ICE or Chao2 are characterised by the least amount of positive or negative bias for small sampling sizes, see also Fig. 2). These estimators are however significantly correlated with the observed number of species ($r_{Sobs\text{-}ICE,24}$ = 0.845, P<0.001; $r_{Sobs\text{-}Chao2,24}$ = 0.753 ,P<0.001), so the use of S_{obs} is advisable since it includes no bias due to the estimating algorithm, only bias due to different activity or sampling probabilities of the species.

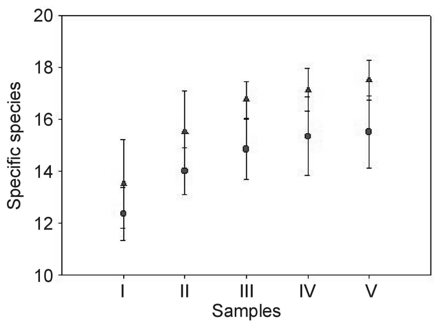


Fig. 3. Collector curves from two types of grey dunes patches in the coastal dune landscape (dots: moss dominated dune; triangle: short dune grasslands). Mean values of the oberved number of species and standard deviations are given.

DISCUSSION

Our results indicate that appropriate methods for estimating regional habitat total species richness severely depend on the structure of the collector curve. In our case study with spider data from the Flemish coastal dunes, the best fit to the collector curve of all species was always reached by a non-asymptotic function, while for the specific species from grey dunes, the asymptotic hyperbolic curve fitted the best. All specific species within the Flemish coastal dune region can be found by using a limited amount of pitfall traps, which should, of course, be randomly placed in the study area.

Because specific species were defined on basis of their known habitat preference from literature (Hänggi et al. 1995; Maelfait et al. 1998; Bonte et al. 2002) and not solely from the indicator species analysis conducted for the Flemish coastal dune spider assemblages (Bonte et al. 2002), we are sure that increasing sampling effort would not increase the number of typical species, because the list is limited and more than 95% of them are already found.

The non-asymptotic model for regional habitat species richness indicates that a large sampling effort is necessary if one aims to estimate the total species richness. This means biologically that in one habitat type, extra species still can be found if the sampling effort increases. These should however, be all non-typical, more eurytopic species from adjacent habitats because the collector curve of the specific species is asymptotic (hyperbolic). This is in accordance with the large variation in community structure in fragmented dune habitats due to the presence of species from adjacent habitats (Bonte et al. 2002). In an analogue study, Samu & Lövei (1995) were able to describe the spider collector curve from an apple orchard as an asymptotic function, derived from the island biogeography theory. Although this result is apparently contrasting, we should bear in mind that they sampled an orchard, situated in agricultural land during a short period in spring, in which all captured species can be regarded as typical. Because of the less fragmented character of this habitat and the related matrix, increasing sampling effort (more pitfall traps) did not result in extra-species. The short sampling period additionally decreased the chance that species from neighbouring, different habitats invaded the orchard (Samu & Lövei 1995). In our study expected species richness varied largely at small sample sizes and became more consistent as sample size increased. In contrast to this, less than 30 pitfalls were necessary to reach a reliable estimate of the richness of specific species in dune grassland and moss-dominated vegetation. This probably resulted from the low structural diversity of the habitat (short grasslands) and the related active hunting strategies of the species (Bonte et al. 2000), which ensured high trapping probabilities for almost all species (only myrmecophile species were rarely captured). The fact that fewer pitfalls were necessary to obtain reasonable richness estimates in moss-dominated dunes compared to the grassland (more structure and more species), confirms the influence of habitat structure on the estimates of species richness and the necessary sampling effort (Sunderland et al. 1995).

As a consequence of the non-asymptotic nature of the collector curves for estimating regional habitat species richness, non-parametric estimators should be used for estimating the species richness. Because of an inherent bias of pitfall traps due to species (and population) specific activities, the estimators ICE, Chao2 and the two jackknifing procedures should be preferred. The algorithm of these estimators is based on the occurrence of species in the samples (Colwell 2000). Since the behaviour of the estimator curves differs significantly between habitats and are again non-asymptotical, species richness differences between habitats should be analysed by comparing the shape and behaviour of the different estimator curves, not by comparing one estimate based on equal (even worse, on unequal) number of traps. Colwell & Coddington (1995) also obtained large variation in richness estimates from seed bank samples, dependent of the method and on the number of samples included, and concluded that a comparison of richness estimates certainly depends on the sampled taxon and habitat(s).

At the patch scale, total species richness estimates based on a low amount of traps are certainly highly biased (and hence have a large standard deviation). The number of specific species is however easily collectable with a few traps and can be compared between patches. The observed number of specific species is thus the best (least biased) parameter if one has to compare species richness between several patches, certainly from a conservationist point of view (Bonte et al. in press). Especially the expected deficit in community species richness can be used as an objective evaluation criterion in bio-indication (Bruun 2000).

Finally, we can state that estimating total species richness of typical fragmented coastal dune habitats (Marram dunes, grey dunes and dune grassland) is difficult by using pitfall traps (and probably other sampling methods, too), because of the mosaic arrangement of the different communities and the continuous chance of finding new species as sampling effort increases. Hence, there is no answer to questions like "*how many spider species can be found in a dune grassland?*". Species richness can be estimated only for the specific species (stenotopic xerotherm species) by extrapolation of the collector curve to asymptotic models, which might be useful for bio-indication. Analysing differences in species richness between patches or habitats can best be done by comparing the non-parametric estimator curves between habitats or by comparing the number of observed species per patch. For a low amount of traps, only the Chao2 estimator gives an error estimate and can be used for proper statistical analyses. Inherent to the relative nature of pitfall trapping, no individual based estimation methods should be used. In case of absolute sampling techniques, like quadrat sampling, fenced pitfall trapping and suction sampling (Sunderland et al. 1995), individual based estimates can be calculated and are intrinsically more reliable.

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Appendix. List of the specific xerotherm species, used in the richness analysis with indications of their main habitat(s) in Central Europe (after Hänggi et al. 1995).

Species	Main habitat in Central Europe
Acarthauchenius scurrilis (O.P.-Cambridge, 1872)	coastal dunes / ant nests
Agroeca cuprea Menge, 1873	xerotherm
Agroeca lusatica (L. Koch, 1875)	heathland
Agroeca proxima (O.P.-Cambridge, 1871)	xerotherm
Alopecosa barbipes (Sundevall, 1833)	xerotherm
Alopecosa cuneata (Clerck, 1757)	xerotherm
Alopecosa fabrilis (Clerck, 1757)	xerotherm
Arctosa perita (Latreille, 1799)	xerotherm
Argenna subnigra (O.P. Cambridge, 1861)	xerotherm
Ceratinopsis romana (O.P.-Cambridge, 1872)	xerotherm
Cheiracanthium virescens (Sundevall, 1833)	xerotherm
Clubiona frisia Wunderlich & Schütt, 1995	xerotherm
Drassodes cupreus (Blackwall, 1834)	xerotherm
Drassodes lapidosus (Walckenaer, 1802)	xerotherm
Erigone promiscua (O.P.-Cambridge, 1872)	xerotherm
Euryopis flavomaculata (C.L. Koch, 1836)	chalk/heathland
Hahnia nava (Blackwall, 1841)	xerotherm
Haplodrassus dalmatensis (L. Koch, 1866)	xerotherm
Haplodrassus signifer (C.L. Koch, 1839)	xerotherm
Hypsosinga albovittata (Westring, 1851)	xerotherm
Marpissa nivoyi (Lucas, 1846)	xerotherm
Mastigusa arietina (Thorell, 1872)	xerotherm / ant nests
Metopobactrus prominulus (O.P.-Cambridge, 1872)	xerotherm
Micaria dives (Lucas, 1846)	xerotherm
Ozyptila atomaria (Panzer, 1841)	xerotherm
Pardosa monticola (Clerck, 1757)	xerotherm
Pelecopsis nemoralis (Blackwall, 1841)	coastal dunes
Peponocranium ludicrum (O.P.-Cambridge, 1861)	chalk/heathland
Philodromus fallax Sundevall, 1833	coastal dunes
Phlegra fasciata (Hahn, 1826)	xerotherm
Sitticus saltator (O.P.-Cambridge, 1868)	coastal dunes
Thanatus striatus C.L. Koch, 1845	xerotherm
Trichopterna cito (O.P.-Cambridge, 1872)	xerotherm
Thyphochrestus digitatus (O.P.-Cambridge, 1872)	xerotherm
Xerolycosa miniata (C.L. Koch, 1834)	coastal dunes
Xysticus erraticus (Blackwall, 1834)	xerotherm
Xysticus sabulosus (Hahn, 1832)	coastal dunes
Zelotes electus (C.L. Koch, 1839)	xerotherm
Zelotes longipes (L. Koch, 1866)	xerotherm