



## Multi-stage cluster sampling for estimating average species richness at different spatial grains

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**Abstract:** A multi-stage cluster sampling is proposed for quantifying and monitoring plant species richness at multiple spatial grains over large spatial extents. An unbiased estimator of average species richness at different grains and a conservative estimator of its sampling variance are obtained in a complete design-based framework, i.e., avoiding any assumption about the ecological community under study. An application to the Nature Reserve "Lago di Montepulciano" demonstrates that the proposed strategy may accomplish practical advantages and quite satisfactory levels of accuracy.

**Abbreviations:** MP – Macroplot, PL – Plot, SP – Subplot.

### Introduction

Ecologists and conservation managers are pressed by the need of assessing the effectiveness of nature reserve networks to preserve biodiversity at large spatial extents, such as nature reserves or parks (Polasky et al. 2000, Rodrigues and Gaston 2002, Bassett and Edwards 2003, Araujo 2004, Rodrigues et al. 2004, Rothley and Rae 2005). However, large, multi-scale and spatially extensive investigations require abundance of funding and time, resources that are scarcely available (Barnet and Stohlgren 2003). In these cases, species richness data at the plot scale (often referred to as  $\alpha$ -diversity) are used, as well as data on other levels of species diversity, such as the classical  $\gamma$ -diversity and  $\beta$ -diversity (Whittaker 1972).

On large spatial extents, the total number of species is practically unknown even after extensive sampling campaigns, simply because of the uncertainty about the completeness of the species list (see e.g., Bunge and Fitzpatrick 1993, Colwell and Coddington 1994, Palmer 1995, Palmer et al. 2002, Hortal et al. 2006). However, ecologists and conservation managers are interested in estimating the number of plant or animal species, in order to obtain extinction rates or to understand the effects of management practices (Mann 1991, Bunge and Fitzpatrick 1993). Consequently, management decisions are

often taken on the basis of local, small scale data such as plant species richness in sample plots.

In order to detect community changes in response to different stresses or dynamics, an ideal sampling strategy should be able to provide information about different levels of species diversity (i.e.,  $\alpha$ -,  $\beta$ - and  $\gamma$ -diversity) and at different spatial grains (Yoccoz et al. 2001, Ferretti and Chiarucci 2003). However, estimation of species richness cannot be considered as independent from the main components of the spatial scale, considering both grain and extent (Arrhenius 1921, Gleason 1922, Connor and McCoy 1979, Palmer and White 1994, Godfray and Lawton 2001, Cowling et al. 2003, but see Scheiner et al. 2000, Dungan et al. 2002, for a complete discussion of scale partitioning into grain and extent). For this reason, programs aiming at quantifying and/or monitoring species richness and composition should adequately consider spatial scale issues (Kenkel et al. 1989, Liu 1995, Yoccoz et al. 2001, Ferretti and Chiarucci 2003). Strictly speaking, size (i.e., grain) and distribution (i.e., extent) of sampled sites may impact the measures of ecological diversity, accounting for the variation in spatial detected heterogeneity across landscape (Stohlgren et al. 2000, Barnet and Stohlgren 2003).

The present paper proposes the use of a multi-stage cluster sampling with the aim of estimating the average

species richness at multiple spatial grains over large spatial extents. It is worth noting that the paper moves in the framework of the design-based inference, which is solely determined by the characteristics of the sampling scheme and, in contrast with model-based inference, avoids any assumption about the population under study. A revision of the two approaches and their implications in environmental surveys is considered in Gregoire (1998). As pointed out by Sarndal et al. (1992, p. 21), the main attractiveness of the design-based approach is that “*Design-based inference is objective, nobody can challenge that the sample was really selected according to the given sampling design. The probability distribution associated with the design is “real”, not modelled or assumed*”. Accordingly, without the help of assumptions regarding the ecological community under study (e.g., the spatial autocorrelation structure which is necessary to perform kriging), an unbiased estimator of the average species richness at different spatial scales is obtained by means of the well-known Horvitz-Thompson estimation criterion, while a conservative estimator of the sampling variance is obtained by means of the Hansen-Hurwitz criterion (for a complete treatment of these widely applied procedures see, among others, Hedayat and Shina, 1991, Chapter 3 and Thompson, 1992, Chapter 6). Finally, it should also be noticed that, as often in ecological sampling, multi-stage cluster schemes are adopted because of their convenience and practicability rather than for their statistical efficiency. Indeed, the great appeal of cluster schemes is that it is often easier and less expensive to locate and observe a sample of spatial units in the same cluster than to locate and observe the same number of units randomly spread over the study area (Thompson 1992, pp. 123-132).

### Statistical background

Consider a delineated study area, previously partitioned into  $L$  spatial zero-level blocks of equal size. If each block  $l$  ( $l = 1, \dots, L$ ) is partitioned into  $N_1$  primary spatial units of equal size, and if  $y_{lh}$  denotes the value of the interest variable within the primary unit  $h$  ( $h = 1, \dots, N_1$ ) in the zero-level block  $l$ , then the population total over the study area may be written as

$$T = \sum_{l=1}^L \sum_{h=1}^{N_1} y_{lh} \quad (1)$$

and it can be estimated by a one-stage stratified sampling of primary units.

To this purpose, at the first stage, each zero-level block may be partitioned into  $L_1$  spatial one-level blocks of  $M_1 = N_1/L_1$  contiguous primary units and a sample of

units may be selected by randomly choosing a unit per block (see e.g., the upper part of Fig. 1 in which a zero-level block constituted by  $N_1 = 32$  primary units is partitioned into  $L_1 = 4$  one-level blocks of  $M_1 = 8$  primary units and a unit is randomly selected in each one-level block  $l$ ). Thus, denote by  $S_l$  the set of indices identifying the primary units selected within the zero-level block  $l$ . From elementary probabilistic considerations, the probability that a primary unit enters the sample (usually referred to as ‘*first-order inclusion probability*’) invariably equals  $M_1^{-1}$ . Accordingly, the one-stage Horvitz-Thompson estimator of  $T$  turns out to be

$$\hat{T}_1 = M_1 \sum_{l=1}^L \sum_{h \in S_l} y_{lh} \quad (2)$$

with variance

$$V(\hat{T}_1) = M_1(M_1 - 1) \sum_{l=1}^L \sigma_{l(k)}^2 \quad (3)$$

where  $\sigma_{l(k)}^2$  denotes the variance of the  $y_{lh}$ -s within the one-level block  $k$  ( $k = 1, \dots, L_1$ ) in the zero-level block  $l$ . Moreover, since only one primary unit is selected within each one-level block, the probability that a couple of primary units enter the sample (usually referred to as ‘*second-order inclusion probability*’) is obviously zero for each couple of units in the same one-level block. Unfortunately, the presence of some second-order inclusion probabilities equal to zero precludes the unbiased estimation of the sampling variance (see e.g., Hedayat and Shina 1991, pp. 49-50). When the design does not ensure invariably positive second order inclusion probabilities, an estimator usually referred to as Hansen-Hurwitz estimator is widely adopted. Wolter (1985) proves the estimator to be conservative for most fixed-size designs. In this framework the Hansen-Hurwitz variance estimator reduces to

$$v_1^2 = \frac{1}{LL_1(LL_1 - 1)} \sum_{l=1}^L \sum_{h \in S_l} (LN_1 y_{lh} - \hat{T}_1)^2. \quad (4)$$

When the quantification of the interest variable is needed at smaller spatial grains, each primary unit may be partitioned into  $N_2$  secondary spatial units of equal size. Thus, denote by  $y_{lhj}$  the value of the interest variable within the secondary unit  $j$  ( $j = 1, \dots, N_2$ ) in the primary unit  $h$  in the zero-level block  $l$ , where

$$y_{lh} = \sum_{j=1}^{N_2} y_{lhj}. \quad (5)$$

Accordingly, the population total over the study area may be rewritten as

$$T = \sum_{l=1}^L \sum_{h=1}^{N_1} \sum_{j=1}^{N_2} y_{lhj}, \quad (6)$$

and it may be estimated by a two-stage stratified sampling of secondary units. To this purpose, at the second stage, each selected primary unit  $h \in S_l$  may be partitioned into  $L_2$  spatial two-level blocks of  $M_2 = N_2 / L_2$  contiguous secondary units and a sample of  $L_2$  units may be selected by randomly choosing a unit per two-level block (see e.g., the middle part of Fig. 1 in which a primary unit constituted by  $N_2 = 81$  secondary units is partitioned into  $L_2 = 9$  two-level blocks of  $M_2 = 9$  secondary units and a unit is randomly selected in each two-level block). Then, denote by  $S_{lh}$  the set of indexes identifying the secondary units selected within the primary unit  $h$  in the zero-level block  $l$ . Since the first-order inclusion probabilities of secondary units are invariably equal to  $(M_1 M_2)^{-1}$ , then the two-stage Horvitz-Thompson estimator of  $T$  turns out to be

$$\hat{T}_2 = M_1 M_2 \sum_{l=1}^L \sum_{h \in S_l} \sum_{j \in S_{lh}} y_{lhj} \quad (7)$$

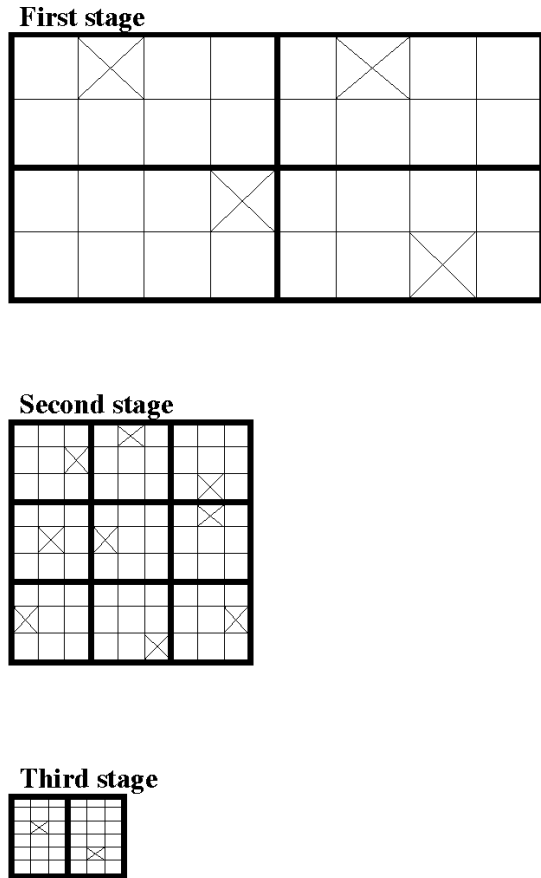
with variance

$$V(\hat{T}_2) = M_1(M_1 - 1) \sum_{l=1}^L \sum_{k=1}^{L_1} \sigma_{l(k)}^2 + M_1 M_2 (M_2 - 1) \sum_{l=1}^L \sum_{h=1}^{N_1} \sum_{k=1}^{L_2} \sigma_{lh(k)}^2 \quad (8)$$

where  $\sigma_{lh(k)}^2$  denotes the variance of the  $y_{lhj}$ -s within the two-level block  $k$  ( $k = 1, \dots, L_2$ ) of the primary unit  $h$  in the stratum  $l$ . Once again, the second-order inclusion probabilities for secondary units in the same two-level block are zero, thus precluding the unbiased estimation of (2). In this case, the Hansen-Hurvitz variance estimator reduces to

$$v_2^2 = \frac{1}{LL_1 L_2 (LL_1 L_2 - 1)} \sum_{l=1}^L \sum_{h \in S_l} \sum_{j \in S_{lh}} (LN_1 N_2 y_{lhj} - \hat{T}_2)^2 \quad (9)$$

In general, the procedure may be straightforwardly generalized to an arbitrary number of stages (see e.g., the lower part of Fig. 1 in which a secondary unit constituted by  $N_3 = 36$  third-level units is partitioned into  $L_3 = 2$  three-level blocks of  $M_3 = 18$  third-level units and a unit is randomly selected in each three-level block). It is worth noting that any further stage  $m$  involves a further term in the variance expression regarding the selection of  $m$ -level units within the previously selected units of lower order. Moreover, since stratification into blocks of contiguous units eliminates the between-block variability, the selection of an  $m$ -level units per block is more efficient than their completely random selection. Finally, owing to the initial stratification into  $L$  spatial zero-level blocks, the survey proceeds as a series of  $L$  independent surveys performed within each block, in such a way that the resulting estimate may be viewed as the sum of  $L$  independent es-



**Figure 1.** Graphical representation of a three-stage sampling scheme in which each zero-level block is partitioned into one-level blocks of primary units, each primary unit is partitioned into two-level blocks of secondary units and each secondary unit is partitioned into three-level blocks of third-level units. Crossed quadrats denote the selected units.

timates. Thus, from the Central Limit Theorem, for  $L$  sufficiently large, conservative confidence intervals for  $T$  may be constructed by using the appropriate quantiles of the standard normal distribution.

**Worked example**

The sampling strategy described above was adopted in the framework of the development of a program to monitor plant species diversity within a network of protected areas in the Siena Province, central Italy. The Nature Reserve “Lago di Montepulciano” (11° 54’ 54’’ E, 43° 05’ 50’’ N, datum ED50) was used for implementing and testing the sampling strategy. The Reserve (about 470 ha) is centred on a shallow lake (about 100 ha), in an area that was once a much larger wetland that dominated this part of Italy until the Medicean period (about 16<sup>th</sup> century). Because of its size and location, the area represents

one of the most important wetlands of central Italy, especially for aquatic birds and plants. Different types of marshes, dominated mostly by *Phragmites australis* and *Carex* spp. cover about 280 ha.

The sampling scheme was based on spatial units of different sizes in order to get estimates of the average species richness at different grains. The spatial grains adopted were 1 m<sup>2</sup> and 100 m<sup>2</sup>. The differently sized units were sampled by a multi-stage cluster scheme, with stratifications performed at each stage, as described in the previous section.

Referring to the above notations, the whole study area was enveloped into  $L = 31$  zero-level blocks of size 500 m × 500 m (25 ha each), derived from the 1 km × 1 km UTM (ED50) grid (see Fig. 2.a). Each zero-level block  $l$  ( $l = 1, \dots, 31$ ) was partitioned into  $N_1 = 25$  primary units of size 100 m × 100 m (1 ha), which were referred to as macroplots (MP, Fig. 2.b). Each MP  $h$  ( $h = 1, \dots, 100$ ) in the zero-level block  $l$  was partitioned into  $N_2 = 100$  secondary units of size 10 m × 10 m (100 m<sup>2</sup>), which were referred to as plots (PL). Finally, each PL  $j$  ( $j = 1, \dots, 100$ ) of the MP  $h$  in the zero-level block  $l$  was partitioned into  $N_3 = 100$  third-level units of size 1 m × 1 m (1 m<sup>2</sup>), which were referred to as sub-plots (SP). Accordingly, three finite populations of 775 MPs, 77,500 PLs and 7,750,000 SPs were defined. However, since a large portion of units in these populations fell outside the area of interest (i.e., the area delineated in Fig. 2.a by a thin line), the target populations were restricted to the PLs and SPs lying within the 467 MPs containing more than 0.5 ha of this area. Practically speaking, the actual study region turned out to be the grey area represented in Fig. 2.c. Thus, the units lying outside the grey area were removed from the survey, in the sense that, if a MP containing less than 0.5 ha of the study area was selected, it was not sampled at finer grains (see Fig. 2.d). Hence, the actual populations to sample were constituted by 467 MPs, 46,700 PLs and 46,700,000 SPs. Notice that we decided *a priori* that the density of spatial units was one larger sampling unit (1 ha) for 25 ha of reference area (nature reserve), but other densities are also imaginable.

Plant species composition was recorded at the two smaller levels (PLs and SPs), since getting exhaustive lists of species at 1 ha grain by exploring the whole spatial unit could be a very time consuming procedure, and potentially not feasible with a reliable degree of certainty (Palmer 1995). Accordingly, statistical estimation of the mean number of species richness at the MP level was not performed.

To accurately locate the PLs in the field, a Trimble GeoExplorer3 GPS receiver was adopted. Obviously, GPS could be used for positioning *in situ* samples with an accuracy of up to 4-5 m, as permitted by these high precision GPS receivers (Read et al. 2003, Gao 2006). Since this may prevent from assigning SPs to the exact plot, the SPs were operationally located by means of metric tapes once located the PL in the field.

In order to roughly assess the powerfulness of the used sampling scheme with respect to the completeness of the obtained species list, a rarefaction curve was computed at MP level by means of the expression (5) by Ugland et al (2003, p. 890). Species rarefaction curves report the increase of detected species as the survey effort increases and are usually applied to measure if a site has been sufficiently sampled. A nearly horizontal curve means that few or no species would be collected by a further sampling effort, while a curve which continues to rise sharply near its end means that many new species are still being expected by further sampling efforts.

The procedure described in the previous section was adopted to estimate the average species richness at 1 m<sup>2</sup> and 100 m<sup>2</sup> spatial grains. Denote by  $y_{lhj}$  the number of species within PL  $j$  in MP  $h$  in the zero-level block  $l$  and by  $y_{lhi}$  ( $i = 1, \dots, 100$ ) the number of species within SP  $i$  in PL  $j$  of MP  $h$  in the zero-level block  $l$ . Moreover, denote by  $U_l$  the set of indices identifying the MPs in the zero-level block  $l$  containing more than 0.5 ha of the study area. The object parameters were the average number of species per PL, say  $\mu_{10}^* = T_{10}^* / 46,700$  where

$$T_{10}^* = \sum_{l=1}^{31} \sum_{h \in U_l} \sum_{j=1}^{100} y_{lhj} \quad (10)$$

as well as the average number of species per SP, say  $\mu_1^* = T_1^* / 4,670,000$ , where

$$T_1^* = \sum_{l=1}^{31} \sum_{h \in U_l} \sum_{j=1}^{100} \sum_{i=1}^{100} y_{lhi} \quad (11)$$

It is worth noting that since

$$y_{lhj} \leq \sum_{i=1}^{100} y_{lhi} \quad (12)$$

i.e., the number of species in a PL was obviously bounded by the sum of the number of species in the included SPs, then  $T_{10}^*$  and  $T_1^*$  were different parameters which had to be estimated by a two-stage sampling of PLs and a three-stage sampling of SPs, respectively.

As to the scheme of sampling adopted to estimate these parameters, at the first stage, a MP was randomly selected within each zero-level block  $l$  (note that in this

case no partition into one-level blocks occurred within zero-level blocks, i.e.,  $L_1 = 1$ ) and the sampling procedure was continued only if the selected MP belonged to  $U_l$ . Subsequently, at the second stage, each selected MP belonging to  $U_l$  was partitioned into  $L_2 = 4$  two-level blocks of 25 contiguous PLs and one PL per block was randomly chosen. Finally, at the third stage, each selected PL was partitioned into three-level blocks of 25 contiguous SPs and a SP per block was randomly chosen. Obviously, the sampling scheme induced a random-size sample, depending on the MP selected in the first stage. However, in order to use the Hansen-Hurvitz variance estimator, which tends to be conservative only in the case of fixed-size designs (see the previous section), it was assumed that  $y_{lhj} = 0$  and  $y_{lhji} = 0$  whenever  $h \notin U_l$ . In this case,  $T^*_{10}$  and  $T^*_1$  were equivalent to their unrestricted counterpart, say

$$T_{10} = \sum_{l=1}^{31} \sum_{h=1}^{25} \sum_{j=1}^{100} y_{lhj} \quad (13)$$

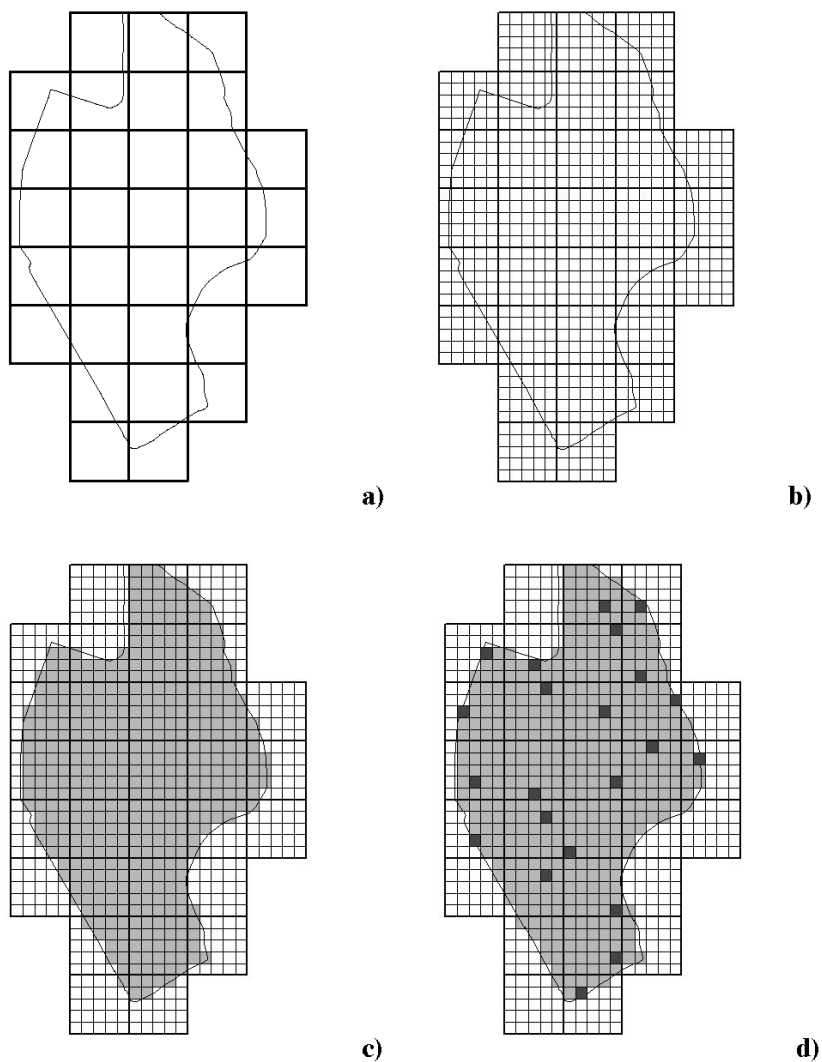
and

$$T_1 = \sum_{l=1}^{31} \sum_{h=1}^{25} \sum_{j=1}^{100} \sum_{i=1}^{100} y_{lhji} \quad (14)$$

while the adopted sampling scheme was viewed as a fixed-size two- and three-stage sampling in which, whenever an MP  $h \notin U_l$  is selected, the subsequent samplings of PLs and SPs gave rise to invariably null values of the interest variable.

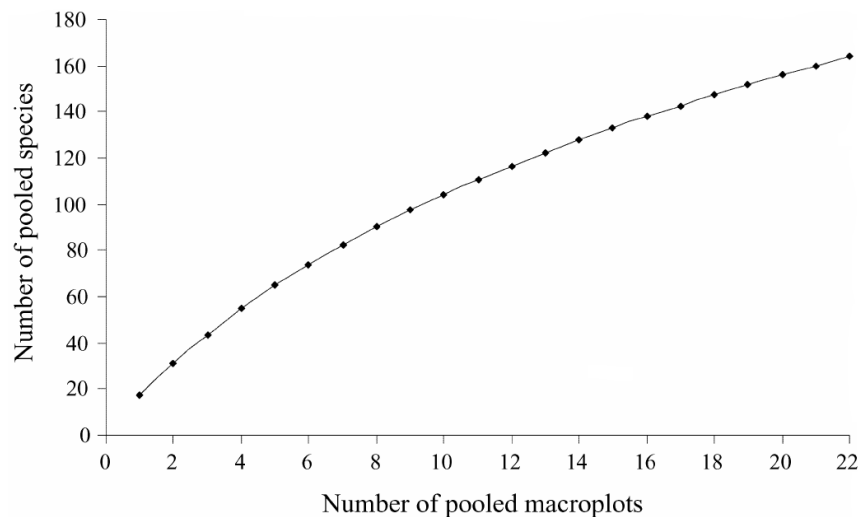
## Results

Although the rarefaction curve resulting from the sample of MPs showed a considerable steepness at its end



**Figure 2.** a) The study area (thin line) was enveloped into  $L = 31$  zero-level blocks of size  $500 \text{ m} \times 500 \text{ m}$  (bold outlined squares); b) each zero-level block was partitioned into  $N_1 = 25$  primary units (MP) of size  $100 \text{ m} \times 100 \text{ m}$  (thin outlined squares); c) since a large portion of the 775 MPs fell outside the area of interest, the target area was restricted to the 467 MPs containing more than 0.5 ha of this area (grey squares); d) if an MP overlaid less than 50 % of the study area, then it was not included in the sample.





**Figure 3.** Species rarefaction curve at MP level.

(see Fig 3), the detected species constituted a floristic list of about 50% of the total flora listed for the same area by a traditional floristic collection (Angiolini et al., in preparation). Even if it should be stressed that in most situations a 50% of total flora accumulates quite fast, the total surface covered by the sampling effort was about 0.16% of the whole nature reserve, thus suggesting a rather good performance of the cluster scheme.

The data obtained from the 352 sampled SPs, nested within the 88 sampled PLs, in turn nested within the 22 sampled MPs, resulted in a total list of 164 species. By using the equations (7) and (9) with  $L = 31$ ,  $N_1 = 25$ ,  $L_1 = 1$ ,  $N_2 = 100$ ,  $L_2 = 4$ , the Horvitz-Thompson estimate of  $T_{10}$  turned out to be  $\hat{T}_{10} = 344,375$  with an estimated sampling variance of  $v_{10}^2 = 2,060,604,039.63$ . Thus, the estimate of the average number of species per PL turned out to be  $\hat{\mu}_{10}^* = 344,375 / 46,700 = 7.37$ , with an estimated variance of  $v_{10}^2 / (46,700)^2 = 0.94$  (standard error  $v_{10} = 0.97$ ). Moreover, by using the three-stage extension of equations (7) and (9) with  $L = 31$ ,  $N_1 = 25$ ,  $L_1 = 1$ ,  $N_2 = 1000$ ,  $L_2 = 4$ ,  $N_3 = 100$ ,  $L_3 = 4$ , the Horvitz-Thompson estimate of  $T_1$  turned out to be  $\hat{T}_1 = 10,437,500$  with an estimated sampling variance of  $v_1^2 = 395,904,356,060.60$ . Thus, the estimate of the average number of species per SP turned out to be  $\hat{\mu}_1^* = 10,437,500 / 4,670,000 = 2.23$ , with an estimated variance of  $v_1^2 / (4,670,000)^2$  (standard error  $v_1 = 0.13$ ). Table 1 reports the estimates of the mean number of species at the two considered spatial grains, together with the corresponding 0.95 confidence intervals and the estimates of the coefficient of variation. The results of Table 1 demonstrate a quite satisfactory level of accuracy compared with the sampling effort and taking into account the conservative nature of the variance estimator which tend to

**Table 1.** Estimates of average species richness at  $1 \text{ m}^2$  and  $100 \text{ m}^2$  spatial grains with 0.95 confidence intervals and estimates of the corresponding coefficients of variation

Spatial scale	Plot	Sub-plot
	$100 \text{ m}^2$	$1 \text{ m}^2$
Range of species richness per unit	0 – 29	0 – 10
Total number of species recorded	164	98
Average species richness per unit (0.95 confidence interval)	7.4 (5.5-9.3)	2.2 (2.0-2.5)
Coefficient of variation	0.13	0.06

overestimate the actual variance. The coefficients of variation at PL level turned out to be of 13% and it drastically reduced to 6% at SP level, showing that the variability of species richness in the population of SPs (around its average value estimated at about 2 species per SP) is much smaller than the variability of species richness in the population of PLs (around its average value estimated at about 7 species per PL). The decreasing of variance from PL to SP populations is also apparent from the ranges of species richness reported in the first row of Table 1.

## Discussion

The collection of species richness data of plants at multiple spatial grains has been one of the basic approaches adopted in classic (Arrhenius 1921, Gleason 1922) and modern vegetation studies (Shmida 1984, Stohlgren et al. 1995, Peet et al. 1998). The sampling schemes adopted in recent years, such as the Whittaker nested vegetation sampling method (see Shmida 1984), the modified-Whittaker nested vegetation sampling

method (Stohlgren et al. 1995), or the nested plot design used by Peet et al. (1998) have long been adopted as standard protocols. However, they were all based on a purposive, rather than probabilistic, location of smaller plots within the larger ones. This precludes the use of any statistical estimation of average species richness at smaller spatial grains, since only a limited part of the population of smaller-size plots is included in the sample. Moreover, the Witthaker plot, as well as the similar sampling schemes, were mainly thought for obtaining estimates of plant species richness at smaller spatial scales within the larger unit (Shmida 1984, Stohlgren et al. 1995, Peet et al. 1998). On the contrary, the sampling scheme presented here was developed for obtaining unbiased estimates of average species richness for the whole study area (such as the whole nature reserve). This can be particularly appreciated for monitoring purposes, when average values for a large extent are needed.

A few methodological problems may arise when using the proposed scheme. In fact, when partitioning the study area into relatively large spatial units, such as the 1 ha MPs used in the worked example, the resulting population is somewhat different from the target population (e.g., the Nature Reserve “*Lago di Montepulciano*” in the worked example), since they have a slightly different perimeter. This is an unavoidable effect when using a reference population of spatial units with fixed size and shape, even if such a problem is negligible when the ratio between the size of the spatial units and the total area under investigation is relatively small. On the other hand, the use of such spatial units represents a basic need, on the strength of the dependence of species richness on plot area (Arrhenius 1921, Gleason 1922, Connor and McCoy 1979, Palmer and White 1994) and shape (see e.g., Stohlgren et al. 1995, Harte et al. 1999a,b, Keeley and Fotheringham 2005). In any case, the issue can be handled by a pragmatic approach, as it was done here, by approximating the reference population to the plots falling for at least 50% within the reference area, or with any other similar assumption.

Since the proposed sampling scheme selects plots without replacement from finite populations of plots, it has the shortcoming of the dependence arising among the selected units and this fact limits the possibility of applying some inferential approaches which could be straightforwardly performed if the plots were randomly and independently placed onto the study area in accordance with the protocol usually referred to as *floating plot sampling* (see De Vries 1986, p. 215). This topic has been detailed by Barabesi and Fattorini (1998, pp. 357-358).

Fortunately, the proposed approach allows for a straightforward assessment of temporal changes in the average species richness, that is one of the basic needs of ecological monitoring. In fact, given the normality of the adopted estimators, the significance of changes can be determined by a simple *t*-test if the two sampling events have been independently performed, or introducing a covariance estimation if the two sampling events were performed on the same spatial units. Obviously, the statistical power of this procedure strictly depends on the structure of the PL and SP populations which, in turn, determine the variability of the estimators and of their difference (see also Legg and Nagy 2006, for a discussion on power analysis).

Finally, it should be clearly noticed that no optimality result (in terms of variance reduction) can be claimed for the proposed strategy. Indeed, it is well known that in a design-based approach the properties of the resulting estimator always depend on the population structure and no uniform optimal sampling strategy may exist (see e.g. Goodambe, 1955, Thompson, 1992, section 9.4). Thus, as pointed out by Fattorini and Barabesi (1998, p. 367), a sampling strategy which may be optimal for one population may not be as precise in other cases. Nevertheless, we firmly underline the operational soundness of the strategy which, at least from the results of the case study, turns out to be satisfyingly accurate. Indeed a sampling effort of 0.16% of the study area provided coefficient of variation below 15% for both the considered spatial grains.

## Conclusion

A number of benefits were recognized for the proposed sampling strategy which: i) collects species data at different spatial grains (1 m<sup>2</sup> and 100 m<sup>2</sup> in our example, but these can be changed according to different needs); ii) gets unbiased estimates of the average species richness at these grains without any assumption about the ecological population under study; iii) effectively reduces the field sampling effort with respect to random allocation of plots.

In a relatively recent paper, Palmer et al. (2002) dealt with the need of developing “more sophisticated statistical models” for “acting with incomplete information” since “conservation decision often need to be made when data are inadequately collected” due to limited resources for the data gathering process, focusing on the data analysis process. Of course we strongly agree with this opinion but we would switch the problem by making a step beyond: based on the current statistical knowledge, could the development of appropriate sampling strategies be able to get reliable estimates of the unknown quantities of

interest from sample (and hence incomplete) information without the use of any model or assumptions?

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