Two-stage estimation of ungulate abundance in Mediterranean areas using pellet group count

**Lorenzo Fattorini · Francesco Ferretti ·
Caterina Pisani · Andrea Sforzi**

Abstract   A design-based strategy for estimating wildlife ungulate abundance in a Mediterranean protected area (Maremma Regional Park) is considered. The estimation is based on pellet group count (clearance count technique) in a set of plots, whose size and number is established on the basis of practical considerations and available resources. The sampling scheme involves a preliminary stratification and subsequent two-stage sampling. In the first stage, large strata (defined through habitat features) are partitioned into spatial units and a sample of units is selected by means of a sampling scheme ensuring inclusion probabilities proportional to unit size, but avoiding the selection of contiguous units. Then, the abundances of the selected units are estimated in a second stage, in which plots are located using a random scheme ensuring an even coverage of the units. In small strata, only the second stage is performed. Unbiased estimators of abundance and conservative estimators of their variances are derived for each strata and for the whole study area. The proposed strategy has been applied since the Summer of 2006 and the estimation results reveal substantial improvement with respect to the previous results obtained by means of an alternative strategy.

Keywords   Stratification · Spatial sampling · Plot sampling · Clearance count ·
Horvitz-Thompson estimation · Empirical inclusion probabilities

L. Fattorini (✉) · C. Pisani
Dipartimento di Metodi Quantitativi, Università di Siena, Siena, Italy
e-mail: fattorini@unisi.it

F. Ferretti
Dipartimento di Scienze Ambientali "G. Sarfatti", Università di Siena, Siena, Italy

A. Sforzi
Museo di Storia Naturale della Maremma, Grosseto, Italy

ⓢ Springer

1 Introduction

Monitoring wild ungulate populations is fundamental for the correct management of ecosystems, because of their ecological as well as management importance (Putman 1996). Thus, identifying a reliable strategy is crucial to effectively estimate population density (Mayle et al. 1999).

The Maremma Regional Park (MRP) is an area of about 10,000 ha located in a coastal part of Central Italy, bearing populations of fallow deer *Dama dama* and roe deer *Capreolus capreolus*, which pose contrasting management problems: the former is locally responsible for damage to crops and requires selective culling, whereas the latter belongs to the native Italian roe deer *C.c.italicus* (Lorenzini et al. 2003), and deserves specific conservation strategies. The hilly morphology and the dense vegetation cover, composed prevalently of Mediterranean shrubwood, prevents biologists from adopting direct counts of deer, leading them to choose an indirect method (Mayle et al. 1999). The pellet group count (based on the counts of faecal pellet groups) is considered the most reliable indirect method (Neff 1968; Mayle et al. 1999). In particular, the clearance count technique (faecal accumulation rate), based on the counts of pellet groups accumulated in sample areas during a time interval (Mayle et al. 1999), has been adopted. During an initial survey, all faecal pellets are removed from sampling areas; a second survey is then carried out to count all pellet groups thus accumulated (Mayle et al. 1999). The time interval between the two surveys should be determined to avoid pellet group disappearance as a result of weathering and the activity of coprophagous insects, and to allow deer to attend the area. This technique is thought to be more accurate than other pellet group count methods (Campbell et al. 2004), because it does not require the knowledge of the faecal pellet group disappearance rate, which could be markedly variable, especially in a Mediterranean area (Massei et al. 1998).

The monitoring program started in the Winter of 2000–01, in an area covering about 7, 000 ha of the whole Park. The sampling strategy adopted to estimate the abundance of fallow deer and roe deer was primarily guided by the time and funds available, which just allowed for a hundred of plots with a 5 m radius to be investigated over the whole area. Attention was mainly focused on the spatial scheme for allocating plots as well as on the estimation criterion, which were chosen in accordance with the paradigms of design-based inference, avoiding any assumptions about the animal populations under study (Fattorini et al. 2004). A preliminary stratification of the study area was performed according to vegetation structure. The purpose was to achieve spatial strata in which animal density was expected to be quite homogeneous. The vegetation analysis provided 12 strata, from small strata of about 10–50 hectares (*abandoned fields*, *fruit groves*, *sand dunes*, *oak wood*) to large strata of thousands of hectares (*cultivated fields*, *Mediterranean shrubwood*). To maintain a homogeneous sampling effort over the whole park, plots were assigned to strata in proportion to their sizes, but ensuring a minimum of 3 plots per stratum, achieving a total number of 117 plots. While plots were randomly and independently placed onto strata smaller than 500 ha and estimation was performed directly, the random placement of a few plots onto the larger strata of thousands of hectares was not judged suitable. As Sutherland (1996) points out, one of the most common census sins is "*counting in one or few large areas rather than a large number of small ones*". Then, in large strata, a two-stage scheme was

carried out. In the first stage, the stratum was partitioned into spatial units (SU) of relatively small size and a sample of SUs was selected by a probabilistic scheme. In the second stage, the plots assigned to the stratum were uniformly partitioned among the selected SUs and abundance estimation was performed in each SU by means of plots, randomly and independently placed onto the unit. Owing to stratification by habitats, animal density was expected to be quite homogeneous within strata, in such a way that abundance was expected to increase with the size of SUs. Thus, size information was adopted at the estimation level using the ratio estimator in conjunction with the Lahiri-Midzuno scheme (Lahiri 1951; Midzuno 1952), a scheme theoretically simple to handle, approaching the properties of simple random sampling without replacement (SRSWOR) while ensuring the unbiasedness of the ratio estimator (Scott and Wu 1981). Unfortunately, the resulting estimates turned out to be highly unreliable, with standard error estimates of about 55% for fallow-deer (providing 12–45 animals per 100 ha as 0.90 confidence interval) and 40% for roe-deer (6–35 animals per 100 ha). The surveys were repeated from Winter 2001–02 to summer 2003 adopting the same methodology even if attempting different stratifications with fewer strata. The abundance estimates and the standard error estimates as well resulted very unstable, with large fluctuations of densities difficult to justify by means of biological/environmental considerations (see Figs. 2 and 3). Moreover, the absence of roe deer was likely unreliable in some open areas of the northern side of the Park, where a presence of roughly 4–8 ind/100 ha was assessed by means of radio-tracking data (Börger et al. 2006; Lovari et al. 2008).

A simulation study was performed to gain more insight into the statistical properties of the sampling strategy (Fattorini and Pisani 2004). Simulation results demonstrated that a large part of the sampling variance was due to abundance estimation within the sampled SUs, which greatly inflated the first-stage variance, mostly in the presence of an aggregated pattern of population units in the study area. Thus, owing to the very clustered distribution of pellet groups, the inadequacy of the sampling effort devoted to second-phase estimation was identified as the relevant cause of the estimation shortcoming in the Park. Accordingly, from the Summer of 2006 to the Winter of 2007/08, an enforced inventory program was planned, increasing the total number of plots from 100 to 200 (Summer 2006–Winter 2006/07) and, finally, to 250 (Summer 2007–Winter 2007/08), but also adopting an alternative scheme to locate plots onto selected SUs or small strata. Instead of random location, a stratified procedure was adopted to provide a more even distribution of plots. Moreover, on the basis of some theoretical and computational advances allowing for the use of novel sampling schemes (Fattorini 2006, 2009), a drawn-by-drawn scheme was adopted in which the SUs were sequentially selected with probabilities proportional to their sizes but avoiding the selection of those SUs contiguous to the previously selected ones.

This paper deals with the theoretical issues of the updated strategy, which are delineated in Sects. 2 and 3. Section 4 deals with the practical aspect regarding the surveys performed since the Summer of 2006, while Sect. 5 contains considerations about the results achieved in the most recent surveys, compared with those achieved in the first part of the monitoring program by means of the strategy described in Fattorini et al. (2004) together with some concluding remarks of a more general nature.

2 One-stage strategy

Following stratification, the survey proceeds as a series of independent surveys performed within each stratum, in such a way that the overall estimate is obtained as the sum of the independent within-strata estimates. Stratification provides noticeable benefits from both theoretical and practical points of view. From a theoretical point of view, stratification reduces the overall variance of the survey by eliminating the between-strata variability while, for a sufficiently large number of strata, it ensures the normality of the overall estimate as the sum of independent random variables and it is likely to improve the proportional relationship between abundance and unit size in order to adopt size as an auxiliary variable. From a practical point of view, stratification ensures a spatialization of the survey results, providing abundance estimates for each stratum.

From the fact that any stratified survey may be viewed as a series of independent surveys performed within strata, only the estimation within a stratum will be considered henceforth. Accordingly, denote by T the abundance of an animal population over a delineated study area $\mathcal{A}$ of size A (e.g. a stratum corresponding to a habitat type). Suppose that before starting the survey the study area is partitioned into N non-overlapping SUs $a_1, \ldots, a_N$ of size $a_1, \ldots, a_N$. Thus, if t_j denotes the abundance within the j-th SU, the overall abundance can be expressed as

$$T = \sum_{j=1}^{N} t_j$$

It is worth noting that the population of SUs actually constitutes an artificial structure which, in turn, determines the population values $t_1, \ldots, t_N$. In some situations, SUs are arbitrarily determined by biologists on the basis of natural or man-made edges which are easily recognizable in the field. Otherwise, tessellation into grids of regularly-shaped, equally-sized SUs can be used by covering the study region with a regularly-shaped enlarged region.

Denote by S a sample of n SUs selected according to a without-replacement sampling scheme inducing first- and second-order inclusion probabilities, say π_j and π_{jh} for $h > j = 1, \ldots, N$. If the abundance in the selected SUs were recorded without error and the first-order inclusion probabilities were known at least for the selected SUs, the Horvitz-Thompson (HT) estimator

$$\hat{T} = \sum_{j \in \mathsf{S}} \frac{t_j}{\pi_j} \tag{1}$$

would have expectation $E_1(\hat{T}) = T$ and variance

$$V_1(\hat{T}) = \sum_{j=1}^{N} \frac{1 - \pi_j}{\pi_j} t_j^2 + \sum_{j=1}^{N} \sum_{h>j} \frac{\pi_{jh} - \pi_j \pi_h}{\pi_j \pi_h} t_j t_h$$

where E_1 and V_1 denote expectation and variance with respect to all the possible samples S which can be selected in the first stage.

As to the scheme to be used for selecting SUs, SRSWOR may prove to be an inefficient design. Indeed, adjacent SUs are often more alike than SUs that are far apart, thus giving a poor contribution to the sample information. This problem is well recognized in a model-based setting as the *spatial autocorrelation* problem. In order to handle the presence of spatial autocorrelation in a design-based approach, Fattorini and Ridolfi (1997) suggest modifying SRSWOR in such a way that, at each of the n drawings, the selection of those SUs that are adjacent to the previously-selected ones is precluded. Moreover, when SUs are of equal size, the population variance simply measures the spatial variation of abundance across the study area. On the other hand, when SUs are of different sizes, the population variance is inflated by the variation in the size of SUs. Since abundance is likely to increase with size, a suitable solution may be the use of a ΠPS design in which the first-order inclusion probabilities are proportional to the sizes of the SUs. That may be straightforwardly performed by adopting one of the 43 fixed-size sampling schemes listed in Brewer and Hanif (1983). However, the presence of unequally-sized SUs is likely to concur with the presence of spatial autocorrelation. Thus, in order to take into account both these problems, the scheme by Barabesi et al. (1997), henceforth referred to as the *BFR scheme*, has been adopted in this framework. The authors suggest modifying the sampling scheme proposed by Skalski (1994) in which, at each of the n drawings the probability of selecting an SU is proportional to its size, by vanishing the selection probabilities of those SUs that are adjacent to the previously-selected ones. Thus, at the first drawing the SUs are selected with probabilities $\tau_1(j) = a_j/A (j = 1, \ldots, N)$. Subsequently, at the i-th drawing $(i = 2, \ldots, n)$, conditional on the first $i - 1$ selected SUs $j_1, \ldots, j_{i-1}$, the remaining $N - i + 1$ SUs are selected with probability

$$\tau_i(j|j_1, \ldots, j_{i-1}) = \begin{cases} 0 & \text{if } j \in C(j_1, \ldots, j_{i-1}) \\ \frac{a_j}{A - a_{j_1} - \cdots - a_{j_{i-1}} - A(j_1, \ldots, j_{i-1})} & \text{otherwise} \end{cases}$$

where $C(j_1, \ldots, j_{i-1})$ denotes the set of SUs which are contiguous to at least one of the $i - 1$ SUs $j_1, \ldots, j_{i-1}$, while $N(j_1, \ldots, j_{i-1})$ and $A(j_1, \ldots, j_{i-1})$ denote the number and total size of these SUs, respectively. The design exists providing that $N - i + 1 > N(j_1, \ldots, j_{i-1})$ for each $i = 2, \ldots, n$. The second-order inclusion probabilities obviously vanish for contiguous SUs. The case of equally-sized SUs is trivially handled using $a_j = A/N \quad (j = 1, \ldots, N)$ in the previous expression.

While the BFR scheme is easy to implement, the HT estimator (1) is inapplicable. Indeed, closed expressions for the first-order inclusion probabilities are lacking while their practical computation involves enumerating all the possible samples and all the orderings in which SUs enter the samples. Thus, the computation becomes prohibitive even for moderate values of N and n. However, since the BFR scheme does not depend on any unknown characteristics of the population, the sample selection can be independently replicated enough times and the inclusion probabilities can be estimated on the basis of the proportion of times in which the SUs enter the selected samples. Fattorini (2006) suggests adopting these estimates (which will be referred to as the

empirical inclusion probabilities) in the HT statistic and in the corresponding variance estimators instead of their true counterparts.

Accordingly, suppose that a sample S of n SUs is selected from $a_1, \ldots, a_N$ by means of the BFR scheme and the selected SUs are subsequently visited to quantify the abundance within. Moreover, quoting from Fattorini (2006), suppose that further M samples of SUs are independently selected from $a_1, \ldots, a_N$ by repeating the BFR scheme. Then, an invariably positive estimator of π_j is given by

$$p_j = \frac{M_j + 1}{M + 1}, \quad j = 1, \ldots, N$$

where M_j is the number of times the j-th SU enters the M samples Since, as $M \to \infty$, the empirical inclusion probabilities constitute consistent estimators of the theoretical counterparts, then a suitable modification of the HT estimator is given by

$$\hat{T}_M = \sum_{j \in S} \frac{t_j}{p_j} \tag{2}$$

which will be referred to as the *empirical HT estimator*. Fattorini (2006) proves that $\hat{T}_M$ is asymptotically ($M \to \infty$) equivalent to $\hat{T}$. Indeed, $\hat{T}_M$ is asymptotically unbiased, in the sense that

$$\left| E_{1M}(\hat{T}_M) - T \right| = O(M^{-1}) \tag{3}$$

and its mean squared error converge to the variance of $\hat{T}$, in the sense that

$$\left| \mathrm{MSE}_{1M}(\hat{T}_M) - V_1(\hat{T}) \right| = O(M^{-1})$$

where $\mathrm{MSE}_{1M}(\hat{T}_M) = V_{1M}(\hat{T}_M) + \left\{ E_{1M}(\hat{T}_M) - T \right\}^2$ and E_{1M} and V_{1M} denote expectation and variance with respect to both the first phase of sampling and the estimation of the inclusion probabilities by the M replicated samples. Moreover, as to the variance estimation, no unbiased estimator exists for $V_{1M}(\hat{T}_M)$ since the second-order inclusion probabilities vanish for contiguous SUs. In this case Fattorini (2006) proves that the empirical counterpart of the Hansen-Hurvitz variance estimator, say

$$\hat{V}_M^2 = \frac{1}{n(n-1)} \sum_{j \in S} \left(\frac{n t_j}{p_j} - \hat{T}_M \right)^2$$

tends to be asymptotically conservative, in the sense that

$$\left| E_{1M}(\hat{V}_M^2) - V_{1M}(\hat{T}_M). \right| = O(M^{-1}) + \Delta$$

where, as argued by Wolter (1985, pp. 43–5), the quantity

$$\Delta = T^2 - 2\frac{n}{n-1}\sum_{j=1}^{N}\sum_{h>j}\frac{\pi_{jh}}{\pi_j\pi_h}t_jt_h$$

is positive for most fixed-size designs.

Once the sample S is selected, the practical problem to be faced is to determine M in such a way that the empirical HT estimate is sufficiently near to the actual (but unknown) HT estimate. To this purpose, the BENNET Algorithm proposed by Fattorini (2009) is adopted. The algorithm is described in Appendix 4.

3 Two-stage strategy

In real situations the abundance $t_j (j \in \mathsf{S})$ cannot be recorded without error. Rather, suppose that abundance is simply estimated by means of plot sampling performed on the population of pellet groups accumulated within the SUs over a pre-fixed number d of days.

Denote by x_j the number of pellet groups accumulated in unit j over d days. If φ represents defecation rate (number of pellet groups deposited by an animal per day), the animal abundance within the unit may be expressed as

$$t_j = \frac{x_j}{d\varphi}$$

In biological literature this relation is considered as deterministic (see e.g Overton 1971 and reference therein), so the problem of estimating t_j is essentially one of estimating x_j by means of r_j plots of size b spread over the SU by means of some design.

Then, in accordance with the clearance count protocol, all the plots in the SU are searched for pellet groups which are removed. After d days, the plots are visited a second time and the number of accumulated pellet groups is recorded.

The basic reference scheme to locate plots within the SUs is *random sampling* in which plots are randomly and independently thrown over the SUs. Even if random sampling is commonly suggested and gives rise to straightforward theoretical results, it is likely to produce unsuitable voids (i.e. undetected parts). Therefore, in order to ensure a more even search over the SUs, stratified schemes are usually preferred. One such scheme involves constructing a tessellation of the SU by means of patches, say $q_{j1}, \ldots, q_{jr_j}$, having approximately the same sizes and then selecting a point in each patch. The tessellation is usually performed by covering the SU by r_j rectangles, quadrats or other regular polygons of the same size, each of them containing at least a portion of the SU and then considering the intersections of these polygons with the unit. Obviously, the inner patches coincide with the polygons and have the same size while edge patches may be smaller. Figure 1 provides a graphical representation of the stratified scheme adopted to locate plots within each selected SU.
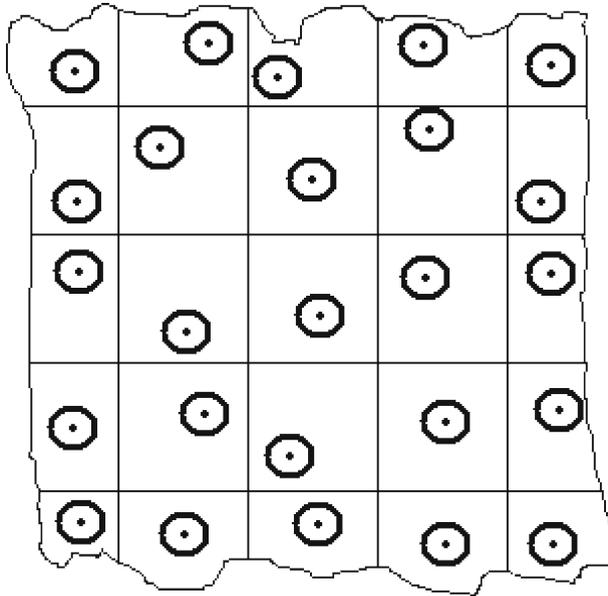
Fig. 1 Graphical exemplification of the scheme adopted to locate plots within the selected SUs. Any SU is partitioned into patches of approximately the same size and a point is randomly selected within each patch. Circular plots with a fixed radius are then constructed around each selected point

If g_{ji} denotes the number of pellet groups accumulated within plot i of unit j over d days and q_{ji} is the size of the corresponding patch, then the weighted sum

$$\hat{x}_j = \frac{1}{b} \sum_{i=1}^{r_j} q_{ji} g_{ji}$$

is proven to be an approximately unbiased estimator of x_j, while

$$\hat{w}_j^2 = \frac{r_j}{r_j - 1} \frac{1}{b^2} \sum_{i=1}^{r_j} \left(q_{ji} g_{ji} - \frac{b\hat{x}_j}{r_j} \right)^2$$

is proven to be a conservative estimator of variance (see Appendix 1). Then, owing to the relation between x_j and t_j, it trivially follows that

$$\hat{t}_j = \frac{\hat{x}_j}{d\varphi} \tag{4}$$

is an approximately unbiased estimator of t_j while

$$\hat{v}_j^2 = \frac{\hat{w}_j^2}{d^2\varphi^2} \tag{5}$$

is a conservative estimator of its variance.

If the $\hat{t}_j$s are used in (2) instead of their true values, the two-stage estimator of abundance turns out to be

$$\hat{\hat{T}}_M = \sum_{j \in \mathsf{S}} \frac{\hat{t}_j}{p_j} \tag{6}$$

where, once again, the empirical inclusion probabilities are determined by means of M replications of the BFR scheme via the BENNET Algorithm. Obviously the variance of (6) will be inflated over the variance of the one-stage estimator (2) by a quantity representing the variability due to the estimation of abundance within the selected SUs.

In accordance with the previous results about the $\hat{t}_j$s, the two-stage estimator $\hat{\hat{T}}_M$ may be proven to have properties similar to the one-stage estimator $\hat{T}_M$, i.e. it is asymptotically ($M \to \infty$) approximately unbiased, while

$$\hat{\hat{V}}_M^2 = \frac{1}{n(n-1)} \sum_{j \in \mathsf{S}} \left(\frac{n\hat{t}_j}{p_j} - \hat{\hat{T}}_M \right)^2$$

tends to be a conservative estimator of its variance in the sense that

$$\left| E_{1M2}(\hat{\hat{V}}_M^2) - V_{1M2}(\hat{\hat{T}}_M) \right| = \Delta + O(M^{-1})$$

where E_{12M} and V_{12M} denote expectation and variance with respect to the two stages of sampling as well as to the estimation of inclusion probabilities (see Appendix 2 and Appendix 3).

It is at once apparent from expression (A6) that the accuracy of $\hat{\hat{T}}_M$ also depends on the numbers of plots assigned to the n selected SUs, which in turn determine the variance of the abundance estimates within these SUs. Unfortunately the variances of the $\hat{t}_j$s do not constitute simple functions of the r_js, in such a way that the minimization of (A6) with respect to the r_js is not straightforward. Thus, the equal assignment $r = R/n$ seems suitable, having at least the practical appeal of ensuring a fixed number of plots over the whole area $\mathcal{A}$.

The main symbols adopted in Sects. 2 and 3, together with their definitions, are listed in Table 1.

4 Application to Maremma Regional Park

The sampling strategy delineated in Sects. 2 and 3 was applied for the first time in the Summer of 2006, when it was decided to increase the number of plots from 100 to 200, taking their size fixed at a 5 m radius. On the basis of previous experience a more effective stratification was performed, avoiding the presence of very small and excessively fragmented strata. The study area was previously partitioned into two main sub-areas: (a) the Northern Sub-Area (NSA), which has an area of about 900 ha (13% of the study area) constituted by open habitats such as crops and pastures with very good visibility, which had served to check the consistency of the pellet count

Table 1 List of definitions for the main symbols adopted in the analytical description of two-stage abundance estimation (Sects. 2 and 3)

Symbol	Definition	Symbol	Definition
$\mathcal{A}$	Study area	$\hat{V}_M^2$	Conservative estimator for the variance of $\hat{T}_M$
A	Size of the study area	$x_1, \ldots, x_N$	Number of pellet groups accumulated within spatial units in d days
T	Abundance on the study area	φ	Daily defecation rate
$\boldsymbol{a}_1, \ldots, \boldsymbol{a}_N$	Spatial units partitioning the study area	r_j	Number of plots to be located onto unit j $(j \in \mathsf{S})$
$a_1, \ldots, a_N$	Sizes of the spatial units	R	Total number of plots
$t_1, \ldots, t_N$	Abundance within spatial units	b	Size of the plots
S	Sample of spatial units	$\boldsymbol{q}_{j1}, \ldots, \boldsymbol{q}_{jr_j}$	Patches partitioning unit j $(j \in \mathsf{S})$
n	Sample size	$q_{j1}, \ldots, q_{jr_j}$	Sizes of patches partitioning unit j $(j \in \mathsf{S})$
π_j	Inclusion probability of unit j	$\hat{t}_j$	Second-stage estimator of abundance within unit j $(j \in \mathsf{S})$
$\hat{T}$	One-stage HT estimator of abundance	$\hat{v}_j^2$	Conservative estimator for the variance of $\hat{t}_j$ $(j \in \mathsf{S})$
p_j	Empirical inclusion probability of unit j	$\hat{\hat{T}}_M$	Two-stage estimator of abundance
$\hat{T}_M$	One-stage empirical HT estimator of abundance	$\hat{\hat{V}}_M^2$	Conservative estimator for the variance of $\hat{\hat{T}}_M$

estimates with those achieved by means of direct counts; (b) the Central-Southern Sub-Area (CSSA), measuring about 6,200 ha (87%) with a massive presence of Mediterranean shrubwood (together with pinewood, ecotone habitats, grassland set-aside and crops) precluding the application of any direct counts.

NSA was partitioned into strata on the basis of the animal density assessed by transect counts carried out in February 2006. Transects were walked in three consecutive days, at dawn, to cover the whole area. The local visibility of deer was exceptionally high, even in broad daylight, because of low vegetation cover and group living habits. Three main zones were identified with substantial differences in their densities. Since animals proved to be markedly sedentary in the area, as also shown by radio-tracking data (Börger et al. 2006), these differences were supposed to be stable and the 3 zones were adopted as strata. On the other hand, CSSA was partitioned into 8 strata according to vegetation structure and spatial contiguity. The identification codes of the 11 strata and their sizes $A_l (l = 1, \ldots, 11)$ are reported in the first two columns of Table 2.

NSA was judged the most problematic to sample, owing to low population density and clumped dispersion of deer (which, as for large herbivores, is higher in open hab-

Table 2 Assignment of plots within strata and within spatial units (Summer 2006)

Sub-area	Stratum	A_l (ha)	R_l	N_l	n_l	r_l
NA	1	98.3	7			
	2	435.4	30	31	6	5
	3	370.3	25	51	5	5
Total NSA		904.0	62			
CSA	4-Mediterranean shrubwood—North	2051.1	45	113	9	5
	5-Mediterranean shrubwood—South	1588.5	35	83	7	5
	6-Pinewood	874.2	20	33	4	5
	7-Ecotone—North	442.3	10			
	8-Ecotone—South	318.0	7			
	9-Cultivated fields—North	241.3	5			
	10-Grassland set-aside	172.8	5			
	11-Cultivated fields—South	572.8	13			
Total CSSA		6260.9	140	6164.8		
Total study area		7164.8	202			

itats, Gerard and Loisel 1995). These factors, added to an inadequate sampling effort, probably generated the unrealistic estimates achieved in 2000/2003 (see Sect. 1). Thus, in order to improve the accuracy for NSA estimates (and to render more effective their comparison with direct methods), about 30% of the plots were assigned to NSA which instead covered just about the 15% of the whole area, achieving a plot density about 3 times greater than that of CSSA.

In order to ensure a homogeneous sampling effort within the sub-areas, a number of plots, say R_l, approximately proportional to the stratum size, was assigned to each stratum l (see column 3 of Table 2). Since 5 strata were of relatively small size (less than 600 ha), in these cases the two-stage procedure was bypassed and the plots were thrown directly onto the strata by means of the tessellation procedure described in Sect. 3. Accordingly, abundance and variance estimation were performed via (5) and (6), respectively. On the other hand, the 5 strata of greater size were partitioned into a number of SUs, say N_l, determined on the basis of natural or man-made edges, in such a way to be readily identifiable in the field. From the resulting population of SUs, samples of size $n_l = R_l/5$ were selected by means of the BFR scheme, in such a way that a constant number of 5 plots was adopted to perform estimation within each selected SU (see Table 2), while since the Summer of 2007 the number of plots has been increased to 7 per SU.

As to the defecation rate, a value of 25 pellet groups/day was established by Massei and Genov (1998), specifically for the fallow deer population of MRP, while a value of 20 pellet groups/day was adopted for roe deer population (cf. Mitchell et al. 1985). As to the practical implementation of the survey, the plots were revisited after 40 days to record the number of accumulated groups. The period between the two visits was suggested by considerations on the disappearance of pellet groups in Mediterranean

areas (Massei et al. 1998). Finally, for each stratum involving two-stage sampling, the computation of the two-stage estimate $\hat{\hat{T}}_M$ and variance estimate $\hat{\hat{V}}_M^2$ was performed by means of the BENNET Algorithm, adopting inequality (A7) and steps of length $L = 1000$. In order to ensure empirical inclusion probabilities near their actual values, the accuracy parameters ε and α as well as the stability parameter δ were set equal to 0.01, while K was set equal to 100. Notwithstanding this severe choice, the estimation of abundance and relative standard errors for all the strata was not problematic, requiring 1 h, 47 min and 44 s of computation time on a notebook with Pentium 4 CPU running at 3.20 GHz.

NSA was also investigated in the Summer of 2006 by means of intensive spotlight counts (Mayle et al. 1999). The surveys were performed by cars spotlighting fields beside roads. Since a large band of fields was spotlighted (67–73%, depending on road conditions and availability), the density calculated considering the maximum number of deer seen in the spotlighted band was extrapolated to the entire area.

For each stratum, Table 3 and Table 4 report the abundance estimates per 100 ha together with the estimate of the percent standard error (% se) and the 0.90-confidence interval (achieved by using the appropriate quantile of the standard normal distribution function) for fallow-deer and roe-deer populations, respectively. Estimation results for NSA and CSSA and for the whole study area are also reported in the tables. For the 3 strata of NSA, values in brackets report the density assessed by spotlight counts. It is worth noting that while the estimates regarding NSA, CSSA and the whole park, as well as the direct estimates achieved in the smaller strata, being sums or linear combinations of independent quantities, are likely to approach the normal distribution, there is no insight about the normality of the two-stage estimates obtained in large strata.

5 Discussion

Figures 2 and 3 display substantial improvement in the accuracy of the density estimate for both populations. Even if estimation remains inadequate for some strata, with estimates of standard errors over 50%, the estimation for the whole park as well as for NSA and CSSA turns out to be quite satisfactory (see Tables 3 and 4). As to the estimation of fallow deer density, the estimate of standard error decreases from the level of 55% achieved in the Winter of 2000–01 to the level of 12% in the Summer of 2006, with the corresponding 0.90 confidence interval which reduces from 12–45 to 12–19 animals per 100 ha. Similarly, as to the estimation of roe deer density, the estimate of standard error decreases from 40% achieved in the Winter of 2000–01 to 22% in the Summer of 2006, with the corresponding 0.90 confidence interval which reduces from 6–35 to 8–18 animals per 100 ha. Moreover, most of the estimates achieved in the 3 strata of NSA are consistent with those obtained by spotlight counts. The effectiveness of the sampling strategy adopted in the Summer of 2006 was confirmed by the subsequent surveys performed in the Winter of 2006–07, the Summer of 2007 and the Winter of 2007–08. The density estimates turn out to be quite stable in both populations (see Fig. 2), together with the estimates of the standard errors which, apart from
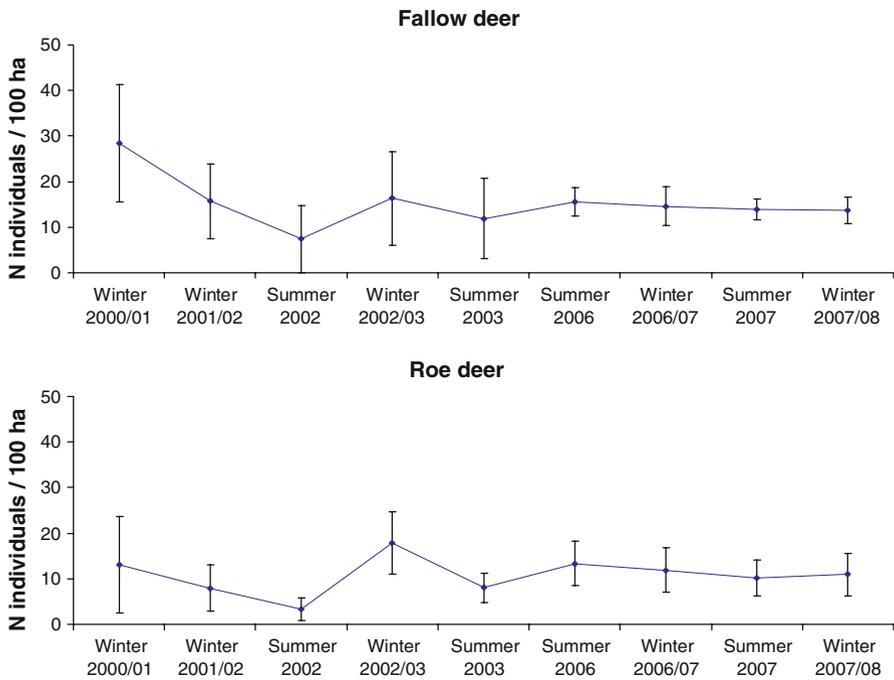
Table 3 Density estimates (individuals per 100 ha) of fallow deer population in Maremma Regional Park (Summer 2006)

Sub-area	Stratum	Density (100 ha)	Se (%)	0.90 Interval
NA	1	27.26(30.53)	20	18.32−36.20
	2	0.10(0.00)	100	0.00−0.26
	3	0.15(4.96)	100	0.00−0.40
Total NSA		3.00(5.35)	19	2.07−3.93
CSA	4- Mediterranean shrubwood—North	17.04	17	12.29−21.79
	5-Mediterranean shrubwood—South	12.45	24	7.55−17.35
	6-Pinewood	11.16	38	4.21−18.11
	7-Ecotone—North	44.15	36	18.08−70.22
	8-Ecotone—South	41.37	50	7.45−75.29
	9-Cultivated fields—North	0.00	–	–
	10-Grassland set-aside	33.54	27	18.69−48.39
	11-Cultivated fields—South	9.66	59	0.31−19.01
Total CSSA		17.32	12	13.91−20.73
Total study area		15.51	12	12.46−18.56

Values in brackets represent spotlight estimates

Table 4 Density estimates (individuals per 100 ha) of roe deer population in Maremma Regional Park (Summer 2006)

| Sub-area	Stratum	Density (100 ha)	Se (%)	0.90 Interval
NA	1	19.78(16.28)	35	8.43–31.13
	2	6.21(6.27)	37	2.44–9.98
	3	2.95(3.82)	50	0.53–5.37
Total NSA		6.31(6.36)	23	3.93–8.69
CSA	4-Mediterranean shrubwood—North	10.91	51	1.78–20.04
	5-Mediterranean shrubwood—South	25.61	41	8.39–42.83
	6-Pinewood	0.00	–	–
	7-Ecotone—North	7.83	56	0.64–15.02
	8-Ecotone—South	42.16	36	17.27–67.05
	9-Cultivated fields—North	9.64	78	0.00–21.97
	10-Grassland set-aside	11.67	38	4.40–18.94
	11-Cultivated fields—South	9.89	35	4.21–15.57
Total CSSA		14.35	23	8.94–19.76
Total study area		13.34	22	8.53–18.15

Values in brackets represent spotlight estimates

some negligible fluctuations, maintain the satisfactory level achieved in the Summer of 2006.

When in Summer 2006 the 2000 monitoring program was revised, all the factors which were presumed to have an influence on accuracy were modified simultaneously:

Fallow deer

Roe deer

Fig. 2 Fallow deer and roe deer density estimates (individuals per 100 ha) in Maremma Regional Park from Winter 2000–01 to Winter 2007–08. *Vertical bars* represent 0.90 confidence intervals
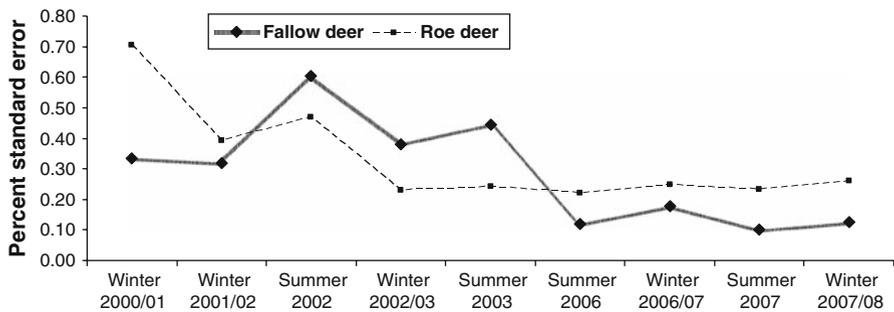
Fig. 3 Percent standard error estimates of fallow deer and roe deer density estimates (individuals per 100 ha) in Maremma Regional Park from Winter 2000–01 to Winter 2007–08

i.e. stratification, the SU selection scheme, plot placement within selected SUs and sampling effort/total number of plots. Even if simultaneously changing all these factors proved effective in rapidly achieving improved estimates, from a purely theoretical point of view it precluded the possibility of determining how much improvement was due to change in the sampling effort rather than to other changes in the sampling scheme. As already noted, the simulation study by Fattorini and Pisani (2004) empirically shows that a large part of the amount of variance in two-stage plot sampling is due to the variance of the second-phase estimation (i.e. estimation within the selected

SUs) which, in turn, decreases with the number of plots therein. Thus, the improvements achieved in the recent surveys seem to be mainly ascribed to the increase in the number of plots, even if the other changes in the sampling scheme have surely contributed to an increase in precision.

In particular, as to the scheme for selecting SUs in the first stage of sampling, it is well known that schemes in which the sampled SUs are spatially balanced (i.e. evenly dispersed over the study area) increase the efficiency of the sampling strategy. In order to ensure spatially balanced samples, Wywial (1996) proposes a design in which the probability of selecting a sample decreases linearly with the number of contiguous SUs in the sample. Similarly, Bryant et al. (2002) prove the existence of a design with equal first-order inclusion probabilities, in which the second-order inclusion probabilities vanish for contiguous units and are otherwise constant. Unfortunately, both these proposals give no insight into the unit drawing chance mechanism corresponding to these designs. Thus the design implementation becomes prohibitive even for moderate values of N and n, since it involves listing all the possible samples and their corresponding probabilities and then selecting one of these samples. Alternatively, *two-dimensional systematic sampling* (2DSS) represents a straightforward way of achieving spatial balance (e.g. Aubry and Debouzie 2000; D'Orazio 2003). The scheme is based on a regular grid of polygons (the SUs) superimposed onto the study area which is subsequently partitioned into regular sub-regions each containing an equal number of polygons. Then the systematic sample is obtained by selecting at random a polygon in one sub-region and then those occupying the same position in the remaining sub-regions. Despite its practical simplicity, 2DSS may be unsuitable in the presence of some spatial regularity, leading to heavy losses of efficiency. In this framework, a suitable solution may be the use of *generalized random tessellation stratified sampling* (GRTSS) which avoids the aligned problems occurring in a complete systematic design. The basic scheme selects points in a two-dimensional continuum but it is also applicable to finite populations of SUs. The technique is based on creating a function mapping two-dimensional space onto a line, thereby defining an ordered spatial address and then randomly ordering the addresses in such a way that systematic sampling along the linear structure provides a spatially balanced sample (Stevens and Olsen 1999, 2003, 2004). While the sampling scheme behind GRTSS might appear quite cumbersome to biologists not trained in complex sampling, the subsequent estimation is straightforward and the inclusion probabilities can be easily accommodated to be proportional to an auxiliary variable (e.g the unit size). As opposed to GRTSS, the BFR draw-by-draw scheme considered in this paper can be easily implemented by biologists but requires computationally intensive estimation owing to the difficulties in determining first- and second-order inclusion probabilities. Obviously, no superiority of one sampling strategy with respect to others can be claimed. In a design-based framework the minimal sufficient statistic (i.e. the unordered set of distinct sampled units) is not complete. Thus, as pointed out by Thompson (2002, Chap. 9), this lack of completeness leads to a lack of optimality results.

As to the general validity of pellet group count estimation, even if the use of faecal counts has become established practice for deer populations, it should be noted that the method suffers from a number of relevant drawbacks. The defecation rate, being

the average number of pellet groups deposited daily by the animals in the population, constitutes an unknown characteristic of the population which must be estimated by means of a sample survey. Accordingly, any defecation rate adopted to convert pellet group counts into an abundance estimate actually constitutes a random variable with an associated sampling variability. Nonetheless, estimation based on faecal counts is customarily performed conditional on the defecation rate estimate, i.e. the defecation rate is assumed to be known and no attempt is made to take into account the impact of its sampling variability in the overall variance. Moreover, as to the determination of the defecation rate, Mayle et al. (1999, p. 88) emphasize that "*Ideally mean defecation rate should be determined for the population under consideration, by following individual animals of different age- and sex-classes and counting the number of defecations within a given period of time. Mean daily defecation rate can then be calculated. This is rarely practicable and so defecation rate determined from captive animals in similar habitats or from literature are most usually used*". Therefore, caution is required when interpreting estimates based on defecation rates obtained in small pens where animals are artificially fed and their movements are reduced, possibly leading to defecation rates different from those occurring in the wild. In accordance with these considerations, we have attempted to reduce this potential source of bias by adopting defecation rates obtained in large enclosures. For fallow deer, we used a winter value of 25 pellet groups/day obtained in an enclosure of 40 ha within our study area, including all the habitats present in MRP (Massei and Genov 1998). For roe deer, in the absence of local data, we used the value of 20 pellet groups/day established by Mitchell et al. (1985) as an average between two enclosures (of 6 and 18 ha) with different environmental conditions. The use of 20 pellet groups/day for roe deer surveys has been also recommended by other authors (e.g. Ratcliffe and Mayle 1992). Finally, defecation rate could vary across seasons since severe variations in climactic conditions (e.g. temperatures ranging from −35 to 35°C as in the study area investigated by Rogers 1987) may determine substantial changes in the feeding activity of deer. Accordingly, the use of a single defecation rate for both winter and summer surveys may constitute an additional source of bias. Even if we could not exclude an across-season variability of defecation in MRP, the variation is expected to be moderate owing to the moderate climactic changes occurring in the park (mean temperatures range from 7 to 24°C and snow is absent).

Appendix 1

Consider an SU $\boldsymbol{a}$ of size a (subfix j is avoided here for simplicity of notation) and denote by G the population of size x constituted by the pellet groups accumulated within $\boldsymbol{a}$ over d days. For each point $u \in \boldsymbol{a}$, denote by

$$\chi(u) = \frac{1}{b} \sum_{k \in \mathsf{G}} z_k(u)$$

where $z_k(u)$ is the indicator function equal to 1 if the k-th pellet group of G lies within the plot of size b centered at u and 0 otherwise. Then, the population size x can be approximately expressed as

$$x \approx \int_a \chi(u) du \tag{A1}$$

The difference between x and the right side of (A1) is due to the pellet groups near the edges of the SU, for which the integral of z_k over a turns out to be smaller than b. However, if b is sufficiently small compared to the size of the SU, the second size of (A1) is slightly smaller than x and the difference can be neglected. If a is partitioned into r patches $q_1, \ldots, q_r$ of size $q_1, \ldots, q_r$ and a plot is randomly selected within each patch, then the plot centers, say $u_1, \ldots, u_r$ are independent random variables uniformly distributed in the corresponding patches.

Accordingly, the weighted sum

$$\hat{x} = \frac{1}{b} \sum_{i=1}^{r} q_i g_i$$

can be rewritten as

$$\hat{x} = \sum_{i=1}^{r} q_i \chi(u_i)$$

in such a way that

$$E_{SS}(\hat{x}) = \sum_{i=1}^{r} q_i E_{SS}\{\chi(u_i)\} = \sum_{i=1}^{r} q_i \int_{q_i} \chi(u) \frac{1}{q_i} du = \int_a \chi(u) du$$

Thus, owing to (A1), $\hat{x}$ is an approximately unbiased estimator of x with variance

$$V_{SS}(\hat{x}) = \sum_{i=1}^{r} q_i^2 V_{SS}\{\chi(u_i)\}$$

$$= \sum_{i=1}^{r} q_i^2 \left[\int_{q_i} \chi^2(u) \frac{1}{q_i} du - \left\{ \int_{q_i} \chi(u) \frac{1}{q_i} du \right\}^2 \right]$$

$$= \sum_{i=1}^{r} q_i \gamma_i - \sum_{i=1}^{r} x_i^2$$

where E_{SS} and V_{SS} denote expectation and variance with respect to the stratified placement of plots over the SU, while

$$x_i = \int_{q_i} \chi(u) du$$

and

$$\gamma_i = \int_{q_i} \chi^2(u) du$$

As to the estimation of $V_{SS}(\hat{x})$, since only one point is selected within each patch, there is no way to achieve unbiased estimators. This is a common problem with stratified spatial sampling when only one point is selected per stratum. Thus, in order to achieve a conservative estimator of $V_{SS}(\hat{x})$, $\hat{x}$ can be rewritten as $\hat{x} = r\bar{y}$ where

$$\bar{y} = \frac{1}{r} \sum_{i=1}^{r} y_i$$

and $y_i = (q_i g_i)/b (i = 1, \ldots, r)$, in such a way that $V_{SS}(\hat{x}) = r^2 V_{SS}(\bar{y})$. Owing to the independence among the g_is (and hence among the y_is),

$$\frac{s^2}{r} = \frac{1}{r(r-1)} \sum_{i=1}^{r} (y_i - \bar{y})^2$$

is a conservative estimator of $V_{SS}(\bar{y})$ in such a way that

$$\hat{w}^2 = rs^2 = \frac{r}{r-1} \frac{1}{b^2} \sum_{i=1}^{r} \left(q_i g_i - \frac{b\hat{x}}{r} \right)^2$$

is a conservative estimator of $V_{SS}(\hat{x})$.

Appendix 2

Denote by $E_2(\bullet|S, \mathbf{p})$ and $V_2(\bullet|S, \mathbf{p})$ the expectation and variance with respect to the second stage conditional on the first-stage sample S and to n-vector of empirical inclusion probabilities $\mathbf{p}$.

From the approximate unbiasedness of the $\hat{t}_j$s it follows that

$$E_2 \left(\sum_{j \in S} \frac{\hat{t}_j}{p_j} \Big| S, \mathbf{p} \right) = \sum_{j \in S} \frac{E_{SS}(\hat{t}_j)}{p_j} \approx \sum_{j \in S} \frac{t_j}{p_j} = \hat{T}_M \qquad \text{(A2)}$$

Thus, from the familiar properties of expectation and from relations (A2) and (3) it follows that

$$E_{12M}(\hat{\hat{T}}_M) = E_{1M}\left\{ E_2\left(\sum_{j \in S} \frac{\hat{t}_j}{p_j} \Big| S, \mathbf{p} \right) \right\} \approx E_{1M}(\hat{T}_M) = T + O(M^{-1})$$

which proves the approximate asymptotic unbiasedness of $\hat{\hat{T}}_M$. Moreover, from the conditional independence of the $\hat{t}_j$s (due to the fact that separate surveys are performed within the selected SUs) it follows that

$$V_2\left(\sum_{j \in S} \frac{\hat{t}_j}{p_j} \Big| S, \mathbf{p} \right) = \sum_{j \in S} \frac{V_{SS}(\hat{t}_j)}{p_j^2} \tag{A3}$$

Thus, from the familiar properties of variance and from relations (A2) and (A3) it follows that

$$V_{12M}(\hat{\hat{T}}_M) = V_{1M}\left\{ E_2\left(\sum_{j \in S} \frac{\hat{t}_j}{p_j} \Big| S, \mathbf{p} \right) \right\} + E_{1M}\left\{ V_2\left(\sum_{j \in S} \frac{\hat{t}_j}{p_j} \Big| S, \mathbf{p} \right) \right\}$$

$$\approx V_{1M}(\hat{T}_M) + E_{1M}\left\{ \sum_{j \in S} \frac{V_{SS}(\hat{t}_j)}{p_j^2} \right\} \tag{A4}$$

The first term of (A4) is the variability due to both the first-stage of sampling and estimation of inclusion probabilities. Quoting from Fattorini (2006, expression A5) the first term turns out to be

$$V_{1M}(\hat{T}_M) = \sum_{j=1}^{N} \left\{ \pi_j E_M(p_j^{-2}) - \pi_j^2 E_M^2(p_j^{-1}) \right\} t_j^2$$

$$+2\sum_{j=1}^{N}\sum_{h>j} \left\{ \pi_{jh} E_M(p_j^{-1} p_h^{-1}) - \pi_j \pi_h E_M(p_j^{-1}) E_M(p_h^{-1}) \right\} t_j t_h \tag{A5}$$

where E_M and V_M denote expectation and variance with respect to the estimation of inclusion probabilities. The second term of (A4) is the variability of the second stage due to the estimation of abundance within the selected SUs. Since the selection of the sample S is independent from the selection of the M samples adopted to estimate the inclusion probabilities, the indicator variables $z_1, \ldots, z_N$ where z_j equals 1 if $j \in S$ and 0 otherwise are independent of $\mathbf{p}$. Accordingly,

$$E_{1M}\left\{\sum_{j\in S}\frac{V_{SS}(\hat{t}_j)}{p_j^2}\right\} = E_{1M}\left\{\sum_{j=1}^{N}\frac{V_{SS}(\hat{t}_j)}{p_j^2}z_j\right\} = \sum_{j=1}^{N}E_1(z_j)E_M(p_j^{-2})V_{SS}(\hat{t}_j)$$

$$= \sum_{j=1}^{N}\pi_j E_M(p_j^{-2})V_{SS}(\hat{t}_j) \tag{A6}$$

Appendix 3

Using the indicator variables $z_1, \ldots, z_N$, the variance estimator $\hat{\hat{V}}_M^2$ can be rewritten as

$$\hat{\hat{V}}_M^2 = \sum_{j=1}^{N}\frac{\hat{t}_j^2}{p_j^2}z_j - \frac{2}{n-1}\sum_{j=1}^{N}\sum_{h>j}\frac{\hat{t}_j}{p_j}\frac{\hat{t}_h}{p_h}z_j z_h$$

from which

$$E_{12M}(\hat{\hat{V}}_M^2) = E_{1M}\left\{E_2\left(\sum_{j=1}^{N}\frac{\hat{t}_j^2}{p_j^2}z_j - \frac{2}{n-1}\sum_{j=1}^{N}\sum_{h>j}\frac{\hat{t}_j}{p_j}\frac{\hat{t}_h}{p_h}z_j z_h \,\middle|\, \mathsf{S}, \mathbf{p}\right)\right\}$$

$$= E_{1M}\left\{\sum_{j=1}^{N}\frac{E_{SS}(\hat{t}_j^2)}{p_j^2}z_j - \frac{2}{n-1}\sum_{j=1}^{N}\sum_{h>j}\frac{E_{SS}(\hat{t}_j)}{p_j}\frac{E_{SS}(\hat{t}_h)}{p_h}z_j z_h\right\}$$

$$\approx E_{1M}\left\{\sum_{j=1}^{N}\frac{V_{SS}(\hat{t}_j^2)}{p_j^2}z_j + \sum_{j=1}^{N}\frac{t_j^2}{p_j^2}z_j - \frac{2}{n-1}\sum_{j=1}^{N}\sum_{h>j}\frac{t_j}{p_j}\frac{t_h}{p_h}z_j z_h\right\}$$

$$= \sum_{j=1}^{N}\pi_j E_M(p_j^{-2})V_{SS}(\hat{t}_j^2) + \sum_{j=1}^{N}\pi_j E_M(p_j^{-2})t_j^2$$

$$- \frac{2}{n-1}\sum_{j=1}^{N}\sum_{h>j}\pi_{jh}E_M(p_j^{-1}p_h^{-1})t_j t_h$$

Thus, from (A5) and (A6), after trivial computations

$$E_{12M}(\hat{\hat{V}}_M^2) - V_{12M}(\hat{\hat{T}}_M) = \sum_{j=1}^{N}\pi_j^2 E_M(p_j^{-2})t_j^2$$

$$-2\sum_{j=1}^{N}\sum_{h>j}\left\{\frac{n}{n-1}\pi_{jh}E_M(p_j^{-1}p_h^{-1}) - \pi_j\pi_h E_M(p_j^{-1})E_M(p_h^{-1})\right\}t_j t_h$$

Quoting from Fattorini (2006, p. 277), the previous difference can be rewritten as

$$E_{12M}(\hat{V}_M^2) - V_{12M}(\hat{\bar{T}}_M) = \Delta + O(M^{-1})$$

Appendix 4

The BENNET Algorithm (Fattorini 2009) has the aim of determining the suitable number of replications to be used for estimating the inclusion probabilities on the basis of the stability of the empirical HT estimate as well as its closeness to the actual (but unknown) HT estimate, checked by means of the Bennet (1962) inequality. More precisely, quoting from Fattorini (2009), the following inequalities hold: given the selected samples S and an arbitrary $\varepsilon > 0$, denote by $\pi_0(S) = \min_{j \in S} \pi_j$, $M_0 = (2 + 2\varepsilon)/\{\varepsilon \pi_0(S)\}$ and

$$c(\varepsilon) = \left(\frac{\varepsilon}{2 + 2\varepsilon} + 1\right) \ln \left(\frac{\varepsilon}{2 + 2\varepsilon} + 1\right) - \frac{\varepsilon}{2 + 2\varepsilon}$$

then, for any $M > M_0$

$$\Pr\left\{\left|\frac{\hat{T}_M - \hat{T}}{\hat{T}}\right| > \varepsilon\right\} \le 2 \sum_{j \in S} e^{-c(\varepsilon)M\pi_j} \tag{A7}$$

$$\Pr\left\{\bigcup_{H=0}^{\infty}\left|\frac{\hat{T}_{M+H} - \hat{T}}{\hat{T}}\right| > \varepsilon\right\} \le 2 \sum_{j \in S} \frac{e^{-c(\varepsilon)M\pi_j}}{1 - e^{-c(\varepsilon)\pi_j}} \tag{A8}$$

The algorithm proceeds by steps, i.e. sequences of L consecutive samples independently selected by means of the sampling schemes adopted to select S. Thus, denote by $\hat{T}_t$, $\hat{M}_t$ and $\hat{\alpha}_t$, the value of $\hat{T}_M$, M_0 and of the right-hand side of inequality (A7) or (A8), computed at the end of step t. Obviously, these quantities are obtained by using the empirical inclusion probabilities $p_j (j \in S)$ achieved at the end of the step via the overall $M = t \times L$ replicated samples instead of the true probabilities $\pi_j (j \in S)$. The algorithm necessitates only the following inputs: (a) the labels identifying the units in the population; (b) the labels of the units in S together with the corresponding values of the interest variable which in the present case are constituted by the $\hat{t}_j$s; (c) an option between the two inequalities (A7) and (A8) and the choice of some parameters necessary to run (see stages 1–4 of the algorithm). Moreover, since the BENNET Algorithm is constructed to work with any replicable sampling scheme, it also needs an algorithm, supplied by the user, with the aim of replicating the sample selection. Obviously, the supplied algorithm must be inclusive of a pseudo-random number generator and of all the parameters needed to replicate the sample selection as, for example, the initial seeds for starting the random number generator. In the present case, the sizes and the neighborhood structures of the SU (i.e. the labels of the contiguous SUs) are necessary to replicate the BFR scheme. The BENNET Algorithm

has been implemented in FORTRAN and the code is available on request from the authors. The main features of the algorithm are described below.

BENNET Algorithm

1: choose $\varepsilon > 0$ and select criterion (A7) or (A8)
2: choose the upper bound α for the right side of inequality (A7) or (A8)
3: choose the step length L
4: choose $\delta > 0$ and an integer K denoting the minimum required number of consecutive steps for which the resulting estimates $\hat{T}_{t-K+1}, \ldots, \hat{T}_t$ have relative differences with $\hat{T}_{t-K}$ invariably less than δ
5: **set** $t = 0$
6: **do**

    6.1: $t = t + 1$
    6.2: generate L samples
    6.3: compute $\hat{T}_t$ on the basis of the overall $M = t \times L$ selected samples
    6.4: **if** $\max \left\{ u : \bigcap_{w=t-u+1}^{t} \left(\frac{\left|\hat{T}_w - \hat{T}_{t-u}\right|}{\hat{T}_{t-u}} < \delta \right) \right\} < K$ **goto** 6.1
    6.5: **if** $M \leq \hat{M}_t$ **goto** 6.1
    6.6: **if** $\hat{\alpha}_t \geq \alpha$ **goto** 6.1
    end do

Practically speaking the algorithm requires the choice of an accuracy parameter ε determining the upper bound of the difference allowed between the HT estimate and its empirical counterpart, as well as the choice of an upper level α for the probability that the relative difference between the two exceeds ε. Typical choices for both ε and α are 0.10, 0.05 or 0.01. As for the choice of the step length L, it should be sufficiently large to ensure substantial changes in the resulting values of the empirical joint inclusion probabilities with respect to those achieved at the end of the previous step. Usually L is chosen to be 1,000 or a multiple of 1,000. Finally, as for the choice of $\delta > 0$ and K, which constitute empirical stability parameters ensuring that the empirical estimates do not vary excessively between steps, suitable choices should be 0.10, 0.05 or 0.01 for δ and 100 or multiples of 100 for K. In this way the computation cannot end until the last $K = 100$ (or multiples) consecutive estimates show relative differences with $\hat{T}_{t-K}$ invariably smaller than 10, 5 or 1%.

The computational effort obviously increases as the accuracy parameters ε and α decrease. Fattorini (2009, Proposition 1 and 2) shows that for a given probability level α, the required number of replications is of order ε^{-2} when working with inequality (A7), while a greater computational effort of order $\varepsilon^{-2} \ln \varepsilon^{-2}$ is required with the more severe inequality (A8). From these findings it is also apparent that the computational effort increases as the joint inclusion probabilities decrease. Moreover, the computational effort may also be massively influenced by the stability parameters $\delta > 0$ and K as well as by other conditions such as population and sample size and the complexity of the sampling scheme, which in turn determines the time necessary to select the replicated samples.

🖄 Springer

References

Aubry P, Debouzie D (2000) Geostatistical estimation of variance for the spatial mean in two-dimensional systematic sampling. Ecology 81:543–553

Barabesi L, Fattorini L, Ridolfi G (1997) Two-phase surveys of elusive populations. In: Proceedings of the statistics Canada symposium 97: new directions in surveys and censuses. Statistics Canada, Ottawa, pp 285–287

Bennet G (1962) Probability inequalities for the sum of independent random variables. J Am Stat Assoc 57:33–45

Börger L, Franconi N, Ferretti F, Meschi F, De Michele G, Gantz A, Coulson T (2006) An integrated approach to identify spatiotemporal and individual-level determinants of animal home range size. Am Nat 168:471–485

Brewer KRW, Hanif M (1983) Sampling with unequal probabilities. Springer, New York

Bryant D, Chang Y, Rodger CA, Wei R (2002) Two-dimensional balanced sampling plans excluding contiguous units. Commun Stat Theory Methods 31:1441–1455

Campbell D, Swanson GM, Sales J (2004) Comparing the precision and cost-effectiveness of faecal pellet group count methods. J Appl Ecol 41:1185–1196

D'Orazio M (2003) Estimating the variance of the sample mean in two-dimensional systematic sampling. J Agric Biol Environ Stat 8:280–295

Fattorini L (2006) Applying the Horvitz-Thompson criterion in complex designs: a computer-intensive perspective for estimating inclusion probabilities. Biometrika 93:269–278

Fattorini L (2009) An adaptive algorithm for estimating inclusion probabilities and performing Horvitz-Thompson criterion in complex designs. Comput Stat 24:623–639

Fattorini L, Ridolfi G (1997) A sampling design for areal units based on spatial variability. Metron 55:59–72

Fattorini L, Pisani C (2004) Variance decomposition in two-stage plot sampling: theoretical and empirical results. Environ Ecol Stat 11:385–396

Fattorini L, Pisani C, Sforzi A (2004) The estimation of wildlife ungulate abundance using sample area surveys: an application to Maremma Regional Park. Stat Method Appl 13:197–212

Gerard JF, Loisel P (1995) Spontaneous emergence of a relationship between habitat openness and mean group size and its possible evolutionary consequences in large herbivores. J Theor Biol 176:511–522

Lahiri DB (1951) A method of sample selection providing unbiased ratio estimates. Bull Int Stat Inst 33:133–140

Lorenzini R, Lovari S, Masseti M (2003) The rediscovery of the Italian roe deer: genetic differentiation and management implications. Ital J Zool 69:367–379

Lovari S, Bartolommei P, Meschi F, Pezzo F (2008) Going out to mate: excursion behaviour of female roe deer. Ethology 114:886–896

Massei G, Bacon P, Genov P (1998) Fallow deer and wild boar pellet group disappearance in a Mediterranean area. J Wildl Manage 62:1086–1094

Massei G, Genov P (1998) Fallow deer (*Dama dama*) winter defecation rate in a Mediterranean area. J Zool Lond 245:209–210

Mayle BA, Peace AJ, Gill RMA (1999) How many deer? A field guide to estimating deer population size. Forestry Commission Field Book n. 18, Edinburgh

Midzuno H (1952) On the sampling system with probability proportional to sum of sizes. Ann Inst Math Stat 3:99–107

Mitchell B, Rowe JJ, Ratcliffe PRR, Hinge M (1985) Defecation frequency in roe deer (*Capreolus capreolus*) in relation to the accumulation rates of faecal deposits. J Zool (London) 207:1–7

Neff DJ (1968) The pellet group count technique for big game trend, census, and distribution: a review. J Wildl Manage 32:597–614

Overton WS (1971) Estimating the number of animals in wildlife populations. In: Giles RH (ed) Wildlife management techniques. The Wildlife Society, Washington pp 403–455

Putman RJ (1996) Ungulates in temperate forest ecosystems: perspectives and recommendations for future research. For Ecol Manage 88:205–214

Ratcliffe PR, Mayle BA (1992) Roe deer biology and management. Forestry Commission Bullettin n. 105, London

Rogers LL (1987) Seasonal changes in defecation rates of free-ranging white-tailed deer. J Wild Manage 51:330–333

Scott A, Wu C (1981) On the asymptotic distribution of ratio and regression estimators. J Am Stat Assoc 76:98–102

Skalski JR (1994) Estimating wildlife population based on incomplete area surveys. Wildl Soc Bull 22:192–203

Stevens DL, Olsen AR (1999) Spatially restricted surveys over time for aquatic resources. J Agric Biol Environ Stat 4:415–428

Stevens DL, Olsen AR (2003) Variance estimation for spatially balanced samples of environmental resources. Environmetrics 14:593–610

Stevens DL, Olsen AR (2004) Spatially balanced sampling of natural resources. J Am Stat Assoc 99:262–278

Sutherland WJ (1996) The twenty commonest censusing sins. In: Sutherland WJ (ed) Ecological census techniques. Cambridge University Press, Cambridge (UK) pp 317–318

Thompson SK (2002) Sampling. Wiley, New York

Wolter KM (1985) Introduction to variance estimation. Springer, New York

Wywial J (1996) On space sampling. Stat Trans 2:1185–1191

Author Biographies

Lorenzo Fattorini is Professor of Statistics at the University of Siena. His field of interest is sampling theory with applications to environmental surveys and ecological diversity analysis. He is Associate Editor of Environmetrics and Journal of Agricultural, Biological and Environmental Statistics and from 2006 to 2009 he has been Coordinator of the Italian Working Group on Sample Survey Methodology.

Francesco Ferretti graduated in Natural Sciences from the University of Naples Federico II in 2005. In 2009 he received the PhD in Sciences and Technologies Applied to the Environment from the University of Siena. Presently, he is working at the Department of Environmental Sciences of the University of Siena, as a post-doc. His research activities are focused on the behavioural ecology, monitoring, management and conservation of meso-large mammals, in the wild.

Caterina Pisani received the PhD degree in Applied Statistics from the University of Florence in 2002 and then joined the Department of Quantitative Methods of the University of Siena where she is Researcher in Statistics from 2006. Her main research interests are concerned with the application of multi-phase designs in environmental surveys for estimating biological population abundance.

Andrea Sforzi graduated in Biology at the University of Perugia, Italy, in 1991. In 1992 he joined the Felidae working group at the Perugia University and, in 1997, the Research Unit of Behavioural Ecology, Ethology and Wildlife Management at the University of Siena. He received the PhD degree in Environmental Sciences from Siena University in 2004. Since 2001 he focused his activity on wildlife management and estimations of wild ungulates populations. Since 1997 he has been charged for the Direction of the Maremma Natural History Museum. His research activities are focused on biogeography and behavioural ecology of wild mammals.