Distance sampling effectively monitored a declining population of Italian roe deer *Capreolus capreolus italicus*

Stefano Focardi, P. Montanaro, R. Isotti, F. Ronchi, M. Scacco and R. Calmanti

Abstract Monitoring rare species of wild ungulates is critical for their conservation management. The Italian roe deer Capreolus capreolus italicus was recently confirmed to be a subspecies in Mediterranean habitats of central and southern Italy. We have monitored this subspecies at Castelporziano, near Rome, since 1988, and detected an abrupt population decline in 2001. We compared distance sampling surveys undertaken before and after the population crash to determine the ability of this method to detect variations in animal density and to investigate which factors may influence the precision of the estimates. We used radio tagged roe deer to evaluate the accuracy of our surveys, comparing distance sampling and mark-resight estimates at the same site, and studying the behavioural reaction of the animals to the presence of an observer. We found that before the crash distance sampling surveys attained a good precision but

that the survey conditions influenced both precision and accuracy. Post-crash surveys were less precise, but the difference in density, before and after the crash was highly significant, indicating the potential of the method to quickly detect density variations and so to allow wildlife managers to react without delay to a crisis. The direct test of distance sampling assumptions showed that estimates were almost unbiased. We conclude that the methodology was successful for monitoring this population and that it may be used in other situations where ungulates are rare and efficient monitoring must be attained with restricted budgets.

Keywords *Capreolus capreolus italicus,* distance sampling, Italy, mark-resight, Mediterranean, population monitoring, roe deer.

Introduction

The monitoring of wildlife populations is central to conservation management. Several methodologies are available for such monitoring (Borchers *et al.*, 2002) but reliable estimates of abundance are conditional on a large sample size, provided there are no other sources of bias, and this implies a large population. Species of conservation interest are, however, often rare and obtaining large samples can be difficult, especially if the budget for research is limited.

The roe deer *Capreolus capreolus* is the most common cervid in Europe (Andersen *et al.*, 1998). Recent research has shown that populations in south-west Europe are genetically different from those elsewhere. Festa (1925) described a subspecies (*C. c. italicus*) that has now been confirmed by genetic evidence (Lorenzini *et al.*, 2003; Randi *et al.*, 2004) using specimens from Castelporziano, central Italy. Genetic variation is correlated with skull structure and body size (Lorenzini *et al.*, 1996; Montanaro *et al.*, 2003).

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Because of their conservation importance at Castelporziano, roe deer have ben monitored since 1988 (Focardi et al., 1996). Using the 1995–1997 surveys Focardi et al. (2002b) showed that distance sampling was a costeffective monitoring method for this population. In distance sampling a standardized survey is carried out along a series of transects. Generally animals become harder to detect with increasing distance from the observer, resulting in fewer detections with increasing distance. The key to distance sampling analyses is to fit a detection function, g(x), to the observed distances, and use it to estimate the proportion of animals missed by the survey (Buckland et al., 2001), assuming that all animals on the line of the transect are detected (i.e. g(0) = 1). The assumptions of distance sampling have been discussed by Buckland et al. (2001).

In this paper we present the results of the long-term monitoring programme and show that the population of roe deer at Castelporziano experienced an abrupt decline in 2000–2001. Comparing surveys before and after the population crash, we retrospectively evaluated whether or not this monitoring method could detect significant differences in density. To improve the design of future surveys we determined which factors influenced the precision of density estimates, as precision has a pivotal role in the detectability of population trends (Gerrodette, 1987). Finally, we evaluated the accuracy of distance sampling for this population and tested the influence of the method's assumptions.

Study area

The extent of the study area (Fig. 1) is different from that reported in earlier work (Focardi *et al.*, 1996; Focardi *et al.*, 2002b) because in 2000 a bordering protected area (Capocotta) was joined to Castelporziano and the dividing fence removed so that ungulates (roe deer, fallow deer *Dama dama*, red deer *Cervus elaphus* and wild boar *Sus scrofa*) could move freely. Thus until 2000 monitoring covered 40.0 km² and it was then extended to 52.4 km²; both values exclude agricultural areas (7.5 km²) partly used as pastures for cattle and horses.

The climate of the area is Mediterranean with a dry summer and rain mostly falling in October–November. Pignatti *et al.* (2001) provided a detailed analysis of the vegetation. The main habitats are holly oak *Quercus ilex* groves (27%), deciduous oak woodlands (34%), and open areas (8.3%) mainly characterized by arid pastures. Commercial stands (21%) are pure or mixed (with *Q. ilex*) woods of domestic pines *Pinus pinea* or cork oaks *Quercus suber*. The remaining land cover is urban areas and sandy shores.

Methods

Long-term monitoring programme

A general count of ungulates was made in the second half of March each year (Focardi *et al.*, 1996; Focardi *et al.*, 2002b). Animals were observed from a variable number of positions throughout the study area. In open areas we used 5 m high observation towers. This method allowed us to record group size and composition (Focardi *et al.*, 1996, 2002b). From 2001 the programme was extended to Capocotta. We performed four replicates of counts until 2000 and five replicates thereafter. To compare the results over 1988–2003 we computed a roe deer index for each survey, which is the mean number of roe deer observed per position and occasion (Vincent *et al.*, 1991).

Distance sampling surveys

Positions of detected animals with respect to the observer were assessed without measurement error using Leitz Geovid binoculars that include a laser range finder (with 1-m error) and an electronic compass (with 1° minimum angle). We used sighting angle and distance to the animal to compute perpendicular distance (Buckland *et al.*, 2001). We only used data for animals whose position could be determined before flushing. To increase encounter rates, transects were covered in early morning or late afternoon, when roe deer are more active.

For 1995–1998 we used 100 transects (each 500 m long) distributed along the forest road network. The compass direction of each transect was randomly selected. In 1995 and 1996 surveys were made in late spring–early summer (13 April–30 June and 11 April–27 June, respectively), whereas in 1997 we surveyed the area twice in winter (28 January–19 March). In 1998 transects were walked in autumn (4 October–5 November).

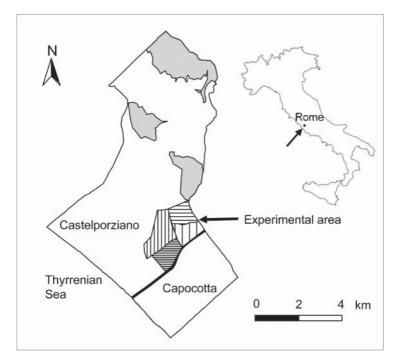


Fig. 1 The study areas of Castelporziano and Capocotta. Fenced areas used for agriculture are in grey. The shading patterns distinguish the four counting zones of the experimental area (see text for details).

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For 2002–2003 52 transects of differing length, with a total distance of 50.5 km, were used. The transects were roughly parallel, in either a north-south or east-west direction, at separations of 700–900 m, provided that a convenient starting point was available and that a suitable choice of the points of passage allowed us to reduce disturbance and thus the chance that animals fled before detection. We used the Geographical Information System ArcView 3.1 (ESRI, 1996) to ensure that the various habitats were covered in proportion to their availability. Surveys were carried out in spring (23 April–24 May) and autumn 2002 (30 November–29 December), and winter (27 January–17 February) and summer 2003 (5 August–4 September).

In January 2000 and December 2001, in an experimental area (4.8 km²; Fig. 1), we captured 32 roe deer and fitted them with Televilt TXH-3 radio collars. A markresight survey was carried out in spring 2003 (3–8 March) when 12 radio collared roe deer remained available. The surveyed area was divided into four zones. In each zone counts were replicated three times for a total of 12 occasions. Observations were made at twilight from 76 spotting locations, i.e. at least five locations per home range. Both tagged and non-tagged deer were recorded and operators working in adjacent locations compared data to eliminate double-observations.

A distance sampling survey was carried out in the same area immediately afterwards (11 March–18 April). Eighteen transects of variable length were used, with a total distance of 47.9 km, and each transect was replicated several times, usually on a different non-consecutive date, alternatively at dawn and dusk.

To determine whether or not animals moved in response to the presence of an operator we fixed, by triangulation, those deer that were in the vicinity of the transect that was to be surveyed, before the distance sampler began work (fix_1) and again after the transect was completely walked (fix_2). The rate of animal displacement was computed from the distance between fix_1 and fix_2 and compared to the observer's speed.

Statistical analyses

For analysis of the roe deer index we used generalized additive modelling (GAM; Hastie & Tibshirani, 1990), which is a flexible method to perform studies on nonlinear time series (Dominici *et al.*, 2002). GAM uses smoothing functions estimated in a non-parametric fashion. We adopted the B-spline for smoothing, an identity link function and a Gaussian error distribution (PROC GAM, release 8.2, SAS Institute Inc., 2000). To reduce arbitrariness in the choice of smoothing parameters we adopted a generalized cross validation to choose the smoothing parameters automatically and to specify the degrees of freedom.

We used DISTANCE (ver. 4.0, release 2) for distance sampling analyses (Thomas *et al.*, 2002). Because our main interest was to detect between-year variation, density was computed per year (there was one survey in each of 1995, 1996 and 1998, and 2 surveys in each of 1997, 2002 and 2003). We used the corrected Aikake Information Criterion (AICc) to select among the different g(x)models by setting the transect width to 85 m, which eliminated the 5% of observations farthest from the centre line of the transects. To improve robustness, the cluster size at 0 distance was evaluated by regressing ln(cluster size) on g(x).

The following estimators were specified as a priori models: uniform, half-normal and hazard-rate keys (adjusted with ≤ 5 terms of the cosine series) for conventional distance sampling and half-normal and hazard-rate keys (adjusted with ≤ 2 terms of the cosine series) for multivariate distance sampling. To reduce the variance of estimates we used covariates that could potentially account for variations in detectability (Thomas et al., 2002). Season (winter, spring, summer and autumn), phases of the life-cycle (non-territorial during October to March, and territorial during April-September), and year were used as factor covariates. As there were many fallow deer in the study area it was possible that their density could affect roe deer detectability, and we therefore used the mean number of fallow deer detected per observation occasion during spring counts as a non-factor covariate.

The AICc selected model was retained if the χ^2 goodness-of-fit statistic was non-significant (default interval selection). The selected g(x) was checked for the presence of a shoulder ($g'(0) \approx 0$), as recommended by Buckland *et al.* (2001).

Provided that animals exhibited equal catchability (zero-truncated Poisson test, Krebs, 1989), mark-resight analysis used the joint hypergeometric estimator. In 'conventional' analysis we estimated population size (NOREMARK; White, 1996) separately for each count zone and, assuming independence of the four zones, pooled the results to obtain a density estimate for the whole experimental area. However, count zones were close and some animals were observed in several zones, and therefore the fraction of residence time in one zone may be <1 (Focardi et al., 2002a) and pooled estimates may be biased. Thus we used a bootstrap approach (Borchers et al., 2002): at each resampling we extracted one set of observations (number of available marked, observed marked and unmarked deer). We replicated this procedure three times, with replacement, to simulate the three occasions, finally computing a pooled value.

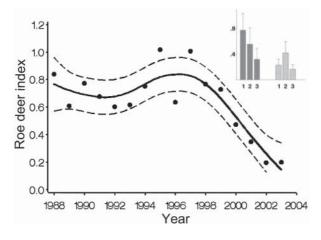


Fig. 2 Variations in the roe deer index from 1988 to 2003, with generalized additive modelling regression (solid line) and 95% confidence limits (dashed lines). In the inset, the index is given separately for Capocotta (dark grey) and Castelporziano (light grey) in 2001, 2002 and 2003 (denoted 1, 2 and 3, respectively) with the corresponding 95% confidence limit (thin vertical bar).

Using 1,000 simulations we computed the median population estimate and used the percentile method to find 95% confidence intervals (Borchers *et al.*, 2002).

To compare the results of different surveys characterized by different variances, when appropriate, we adopted Satterthwaite's approximation to compute the degrees of freedom (which may not be integer) for the Student's t test (PROC TTEST, release 8.2, SAS Institute Inc., 2000). For distance sampling we used the degrees of freedom computed by Distance 4.0. For the mark-resight survey we used the number of observation occasions.

Results

Long-term monitoring programme

The roe deer index remained almost constant over 1988–1999 (Fig. 2) but from 1999 to 2000 there was a marked reduction, and a further decline over 2001–2003, with an average post-1999 reduction of 50%. Both linear (t = -4.2, P = 0.002) and non-linear components ($\chi^2_4 = 28.1$, P < 0.0001) of GAM regression were significant. The index was significantly larger at Capocotta than at Castelporziano in 2001 (Wilcoxon test, z = 5.4, P < 0.0001) but not in 2002 (Wilcoxon test, z = 1.8, P = 0.06) and 2003 (z = 1.7, P = 0.08).

Modelling the detection function

The choice of the g(x) model was based on the comparison of the different models (Table 1). Model 1, with the lowest AICc, had a g(x) that remained unchanged during the study period despite variations in roe deer density and sampling design. The second best model assumes there are three different detection functions: one each for the pre-crash and post-crash periods and one for the experimental survey. However, to estimate a separate detection function for the latter is unsafe because it is based on only 23 observations. The fit is poor (Fig. 3a)

Table 1 The Akaike Information Criteria for the 14 models tested. For conventional distance sampling (CDS) the number of strata and the pooling of surveys (within braces) are indicated. For multiple-covariate distance sampling (MCDS) we report the covariates used in the analysis.

| Model | Sampling | Strata / covariate ¹ | AICc ² | $\Delta AICc^{3}$ | AIC^4 |
|-------|----------|---|-------------------|-------------------|---------|
| 1 | CDS | none | 2100.55 | 0.00 | 2100.50 |
| 2 | CDS | 3 strata: {PRC1,PRC2,PRC3,PRC4}, {POC1,POC2,POC3,POC4}, EXP | 2101.13 | 0.57 | 2100.91 |
| 3 | CDS | 6 strata: PRC1, PRC2, PRC3, PRC4, {POC1,POC2,POC3,POC4}, EXP | 2101.83 | 1.28 | 2101.14 |
| 4 | CDS | 2 strata: {PRC1,PRC2,PRC3,PRC4}, {POC1,POC2,POC3,POC4,EXP} | 2103.90 | 3.35 | 2103.80 |
| 5 | CDS | 5 strata: PRC1, PRC2, PRC3, PRC4, {POC1,POC2,POC3,POC4, EXP} | 2104.25 | 3.70 | 2103.47 |
| 6 | CDS | 2 strata: life-cycle phases | 2104.99 | 4.44 | 2104.87 |
| 7 | MCDS | Fallow deer index | 2105.06 | 4.51 | 2104.81 |
| 8 | CDS | 4 strata: climatic seasons | 2105.87 | 5.32 | 2105.53 |
| 9 | MCDS | 2 factors: pre-crash {PRC1,PRC2,PRC3,PRC4}, and post-crash {POC1,POC2,POC3,POC4, EXP} | 2105.92 | 5.37 | 2105.76 |
| 10 | MCDS | Biological seasons | 2105.95 | 5.40 | 2105.78 |
| 11 | MCDS | Years | 2106.11 | 5.56 | 2105.35 |
| 12 | MCDS | Biological seasons, fallow deer index | 2106.95 | 6.40 | 2106.59 |
| 13 | MCDS | Climatic seasons | 2107.09 | 6.54 | 2106.84 |
| 14 | MCDS | Climatic seasons, fallow deer index | 2108.68 | 8.13 | 2108.21 |

¹PRC1, PRC2, PRC3, PRC4, pre-crash surveys 1–4; POC1, POC2, POC3, POC4, post-crash surveys 1–4; EXP, experimental survey ²AICc, corrected Akaike Information Criterion

³AAICc, difference between AICc of each model and that of Model 1

⁴Uncorrected Akaike Information Criterion

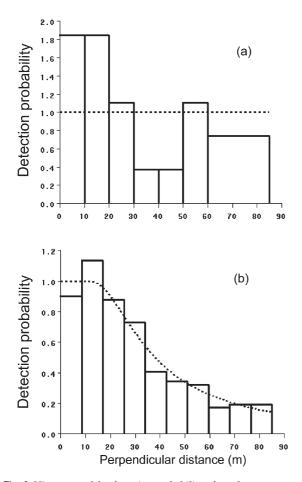


Fig. 3 Histogram of the detection probability of roe deer at increasing perpendicular distances from the transect centre line, and the detection function (dotted line) for (a) the experimental survey in spring 2003 (uniform key) and (b) of the selected model 1 (Table 1; hazard rate key).

and the model tends to underestimate the detection probability close to the line, and thus the density. If we pooled the post-crash surveys (model 3 and 5) the fit was worse than pooling all surveys (model 1). The use of covariates (models 9 and 11) did not improve the fit. The phases of the life cycle appeared to be more likely to influence the detection probability than the climatic seasons (compare models 6 and 8 or models 10 and 13). It seems also that fallow deer abundance may influence roe deer detectability more than season (compare models 7, 10 and 13). Model 6 showed that the detection probability was higher in the territorial (0.573 ± SE 0.03) than in the non-territorial (0.521 ± SE 0.06) phase (Student's test, $t_{203} = 8.8$, P < 0.0001).

The g(x) of model 1 was a hazard rate function, characterized by an effective strip width of 44.9 m, corresponding to a mean detection probability of 0.528 (95% confidence interval, CI, 0.446–0.624). The fitting of the estimated function (Fig. 3b) appears to be good (χ^2_7 =

3.95, P = 0.78), with the shoulder of the distribution suggesting good detectability near the centre line.

Density estimates

Density estimates, using model 1, and adopting a peryear stratification for encounter rate and cluster size, are given in Fig. 4. Pre-crash densities are similar (10-11 deer km⁻²) but it seems that the 1997 survey was negatively biased. The estimates for 2002-2003 were similar, with a density of 2-3 deer km⁻². The 1998 and 2002 estimates were significantly different (Student's test, $t_{273} = 40.5$, P < 0.0001), suggesting an 80% population decrease during this time. The precision was better during the pre-crash period (% coefficient of variation, CV, of 17.7, 13.7, 15.5 and 14.15 for 1995, 1996, 1997 and 1998, respectively) than in the post-crash period (32.8 and 35.9 for 2002 and 2003, respectively) despite an increased effort of two surveys per year during the post-crash period. Mean encounter rates were $0.78 \pm SE 0.05$ and $0.13 \pm SE 0.025$ deer km⁻¹ in the pre- and post-crash periods, respectively.

The confirmatory study

Roe deer did not show ($\chi^2_2 = 0.6$, P = 0.26) heterogeneity in probability of detection during the mark-resight survey. Radio-tagged deer represented 41.4% of the total population. The average probability of detection was 0.59. Conventional mark-resight analysis gave an estimate of 6.1 (95% CI, 5.6–10.3) and bootstrap 5.0 (95% CI, 4.0–6.1) deer km⁻², suggesting a lack of independence among the four count zones (Table 2). However, these estimates were not significantly different (Student's test, $t_{12.2} = -1.6$, P = 0.12), indicating that between-zone dependence was small.

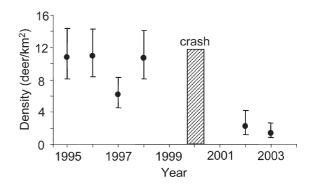


Fig. 4 Estimates of roe deer density for 1995–2003, using distance sampling. From 1995 to 1998 data were collected only at Castelporziano, but at both Castelporziano and Capocotta in 2002–2003.

Table 2 Results of the mark-resight estimate (see text for details) of roe deer in the experimental area (Fig. 1), with the estimated number of roe deer, 95% confidence limits, and the number of radio-tagged animals available during the three replicates of counting.

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Distance sampling estimated 5.36 (95% CI, 3.7–7.7) deer km⁻². The bias of this estimate (CV = 18.6%) was -12% with respect to conventional mark-resight (CV = 37.1%) and +7% for the bootstrap method (CV = 21%). There was no significant difference between the bootstrap mark-resight and distance sampling (Student's test, $t_{25.3} = 1.9$, P = 0.07) and conventional mark-resight and distance sampling (Student's test, $t_{11.6} = -1.1$, P = 0.29).

Distance sampling assumptions

We denoted d_1 (d_2) the perpendicular distances between fix_1 (fix_2) and the transect (Fig. 5a), and expected that if deer performed evasive movements upon the arrival of the observer the difference (d_1 – d_2) would be significantly negative for deer 'near' ($d_1 \le 150$ m) and null for deer 'far' ($d_1 > 150$ m) from the transect, and that there should be a between-group significant difference. Neither groups present a mean (d_1 – d_2) value different from zero (Student's test, far, t = 1.03, P = 0.32; near, t = -1.1, P = 0.29) but the medians of the two groups were significantly different (one-sided Wilcoxon test, z = 1.72, P = 0.04).

To evaluate whether evasive movements were random we computed the distance between the projection of points fix_1 and fix_2 on the transect line (Fig. 5b), expecting that non-random movements would yield significant negative values (the deer fleeing from the observer). In both groups the values were not significantly different from zero (Student's test, far, t = -0.74, P = 0.46, near, t = -0.51, P = 0.62) and there was no between-group difference (one-sided Wilcoxon test, z = 0.30, P = 0.38). The movement of the observer (22.4 ± SE 3.55 m per minute) was *c*. 4 times faster (Student's test, $t_{55} = -21$, P < 0.0001) than that of the roe deer (3.9 ± SE 3.1 m per minute).

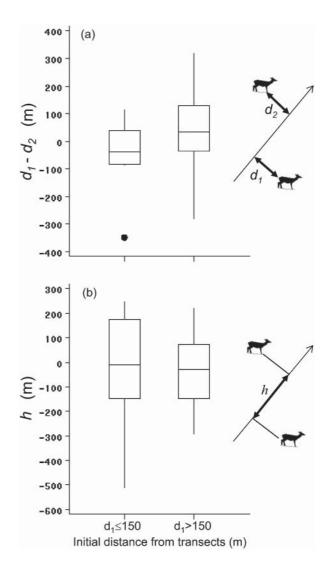


Fig. 5 Box-and-whisker plots of (a) the difference between the perpendicular distances of roe deer from the transect line before (d_1) and after (d_2) a transect was walked, and (b) the difference (h) between the projections of the locations of roe deer onto the transect line before and after a transect was walked, for deer 'near' $(d_1 \le 150 \text{ m})$ and 'far' $(d_1 > 150 \text{ m})$ from the transect. Negative values of (d_1-d_2) indicate that the animal moved away from the transect and negative values of h indicate that animals moved away from the observer.

Discussion

The application of scientific methodologies to conservation problems is often constrained by political or economic considerations. Moreover, when a monitoring programme is long-term methods may be modified because of previous experience or to update them to recent advances. The conditions of work at Castelporziano were typical of an experimental area (since 1988 a scientific commission has cooperated with the Preserve administration; Scarascia-Mugnozza, 2001), allowing us to attain a high level of standardization, but there were decisions (e.g. merging Capocotta and Castelporziano) that obliged us to modify the monitoring design. Finance was a problem but we were nevertheless able to continue the monitoring for 16 years.

Methods that do not incorporate an estimate of detection probability need to be standardized, but this was difficult during the long-term monitoring programme. Nevertheless, the GAM analysis was able to detect the crash, even though it estimated a population reduction smaller than did distance sampling (50% vs 80%, respectively). The long-term monitoring programme indicated that the crash occurred in 1999-2000, and that it started at Castelporziano and spread to Capocotta in 2002-2003. This pattern is confirmed by some drive counts we did in the experimental area in 2000 and 2001, which gave densities of 15.5 and 13.8 roe deer km⁻², respectively, whereas the 2003 experimental survey gave densities of 5-6 roe deer km⁻². Thus we can conclude that the crash started in 2000 and finished in 2003 and that it occurred with some delay at Capocotta and in the experimental area. Vegetation maps (Grignetti et al., 1997; Della Rocca et al., 2001) show that both Capocotta and the experimental area are dominated by mesophilic habitat with a high cover of Carpinus orientalis. This habitat is highly productive and preferred by roe deer (Focardi et al., unpubl. data.), and thus it probably reduced the effect of the crash in this part of the study area. Unfortunately, because of budgetary restraints in 1999, we could not perform distance sampling surveys during the period of the population crash.

To obtain, for the post-crash period, a coefficient of variation comparable to that of the pre-crash period (15%; Buckland *et al.*, 2001) would require a total survey length of 153 km, which corresponds to surveying our transects three times per year. According to Plumtre (2000) the resolution of our study was 41% (for $\alpha = 0.95$) or 58.5% (for $\alpha = 0.99$), for a 15% CV, i.e. to detect a significant difference between two surveys would require a variation of at least 41% (58.5%) in population density.

The confirmatory study gave useful insights into the quality of our distance sampling estimates, and showed that the main assumptions of distance sampling were respected. We could not detect any important differences between mark-resight and distance sampling. The analysis of roe deer behaviour showed that the observer introduced only a small level of disturbance to the deer and moved faster than they did. Hiby (1986) showed that bias is small provided animal movement is less than one half of the observer's speed. The assessment of animal response to the presence of the observer is important for assessing the suitability of the study design, and in this study the detection function had a marked shoulder, suggesting that detectability was high near the line. Roe deer detectability remained constant throughout the study, perhaps a result of the fact that there was little logging in the forest during this time.

This study has shown the potential of distance sampling for the detection of population decline in ungulates of conservation interest although, as expected, the estimates became imprecise as the animals became rare. The roe deer index and the distance sampling estimates were highly correlated (excluding 1997, Pearson correlation, r = 0.92, P = 0.02). One reason for this was the use of a pooled detection function, so that the distance estimates are then effectively mirroring the encounter rates. However, with a variable per-year g(x) (model 11, Table 1) we obtained similar results (Pearson correlation, r = 0.92, P = 0.03).

There are no obvious explanations for the decline of the roe deer at Castelporziano, the population of which is strictly protected. The speed of the decline suggests some kind of disease, but this is an unlikely explanation as sympatric ruminant species were unaffected. A further possible cause could have been interspecific competition, mainly with fallow deer (Focardi *et al.*, in press). Although no strong effect of fallow on roe deer demography was found in the New Forest, UK (Putnam, 1996), fallow deer were considered responsible for the decline of a red deer population in Italy (Mattioli et al., 2003) and a potential for interference competition was demonstrated (Bartos et al., 1996). It is also possible that the establishment of adverse weather conditions, possibly related to global changes, may have negatively influenced the demographic performance of this roe deer population. These possible explanations for the roe deer decline are not necessarily mutually exclusive, and to test them the availability of reliable population indexes and density estimates is of critical importance.

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Roberta Calmanti and Marianne Scacco are students of zoology at the Faculty of Science of the University of Rome 1 and are studying the application of distance sampling to population estimates of fallow deer and wild boar, respectively.

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