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Pellet-count sampling based on spatial distribution: a case study of the European hare in Patagonia

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Resumen. *Las estimaciones de densidad y uso del hábitat a través del conteo de heces se hacen asumiendo una distribución al azar. Presentamos datos de liebre europea* (Lepus capensis) *en el noroeste de Patagonia que muestran que el patrón de distribución de sus heces se ajusta a una distribución agrupada (binomial negativa), y estimamos tamaños mínimos de muestra y varianzas basadas en este modelo. Los tamaños mínimos de muestra fueron mayores y las varianzas menores que los basados en un modelo de disposición al azar. Hacemos recomendaciones para mejorar el método de conteo de heces y métodos similares cuando se puede determinar el patrón de distribución espacial de los individuos a través de un muestreo piloto.*

Abstract. *Estimates of density and habitat use based on fecal-pellet counts have been done in the past assuming a random distribution. We present data on European hares* (Lepus capensis) *in northwest Patagonia showing that the distribution pattern of their pellets fits an aggregated, negative binomial model. We also estimated minimum sample sizes and variances based on this model. Minimum sample sizes were larger and variances were smaller than those based on a random distribution model. We provide recommendations to improve the pellet-count and similar sampling methods when the spatial distribution of the individuals can be determined through a pilot study.*

Introduction

The fecal-pellet-count method has been intensively used to estimate density and habitat use of large and medium-sized mammals (e.g., Amaya 1978, Amaya and Bonino 1980, Amaya et al. 1984, Arnold and Reynolds 1943, Edge and Marcum 1989, Flux 1967, Kufner 1983, 1986, Litvaitis et al. 1985, McClanahan 1986, Taylor and Williams 1956, Wolfe et al. 1982). In all of these studies a random distribution of the pellets was implicitly assumed in the estimation of population variances. Only Kufner (1983) estimated the minimum sample size required to obtain an expected coefficient of variation of the density of pellets, but she also assumed a random distribution. However, most natural populations have a clumped distribution (Rabinovich 1980, Seber 1973), which determines that the estimates of variance and sample size must be based on an aggregated distribution model (Gerard and Berthet 1971).

Regarding the shape of the sampling unit (SU), Amaya and Alsina (1982), Amaya and Bonino (1980), Amaya et al. (1984), Litvaitis et al. (1985), and Wolfe et al. (1982) used a circular one, whereas Kufner (1983) used a rectangular one. In none of these studies was the distribution pattern of the pellets evaluated to establish the optimal size and shape of the SU to be used.

The goal of this study was to improve the fecal-pellet-count method by developing a sampling scheme to estimate the mean and the variance of the number of pellets based on their spatial distribution pattern. This scheme could be used also for sampling any population in which the spatial distribution of individuals can be determined through a pilot study.

We selected the pellets of the European hare (*Lepus capensis*) as a case study. This species was chosen because it is abundant and its pellets can be located easily. Moreover, the methods for estimating European hare abundance need to be improved because this species is of great commercial importance in the area (Cajal 1986, Grigera and Rapoport 1983, Novaro 1991).

Materials and Methods

Study area

Our study area was located on seven ranches: La Rinconada, La Papay, Los Remolinos, Catan Lil, Collun-Co, Cerro de los Pinos, and Aquinco, in the vicinity of the city of Junín de los Andes, province of Neuquén, at 40°S and 71°W. The area is in the Occidental District of the Patagonian Phytogeographic Province (Movia et al. 1982). The vegetation is characterized by a mixed steppe of grass and shrubs. Dominant species are *Mullinum spinosum*, *Senecio* sp., *Stipa* sp., and *Poa* sp.

Topographically the study site consists of great plains 800 to 900 m high, dissected by steep, rugged areas and valleys. In the bottom of the valleys there are humid areas with dense herbaceous vegetation, called "mallines", where dominant species are *Cortadeira araucana*, *Juncus* sp., and *Carex* sp. These are areas of high primary productivity (Movia et al. 1982).

Pellet-count technique

The pellet-count technique (Arnold and Reynolds 1943) consists of counting all pellets found in a series of sampling units (SU) of known size and then counting the number of new pellets dropped after 15 days in these SU. European hare density was estimated using the equation

$$D = \frac{\dfrac{10000\ m^2}{ha\ \bar{X}}}{T\ R\ A} \qquad (1)$$

where D = hare density (individuals/ha), $\bar{X}$ = mean number of pellets per SU in the second count, T = time between first and second count (days), R = defecation rate (number of pellets dropped animal^{-1} day^{-1}), A = area of each SU (m^2)

Several attempts to estimate defecation rate of European hares in the province of Neuquén were unsuccessful, because the animals could not be kept alive in captivity. Thus, defecation rate was assumed to be 410 pellets hare^{-1} day^{-1}, as estimated by Flux (1967) in an area of New Zealand with similar habitat conditions and forage availability.

Survey design

Data from two pilot sampling periods (January-February and June-July, 1988) on La Rinconada Ranch were used to optimize the sampling plan. This design was applied during the following three winters to evaluate hare densities at La Rinconada and the other six ranches.

In our first sampling period (Summer 1988), we established a 2-dimensional rectangular grid of points spaced 10 m apart. At each intersection, two concentric circular SUs of 0.5 and 1 m radius were used. To mark the SU for the second counting of pellets, a wooden stick was fixed in the center of each SU.

In the summer, four habitats were sampled (sample and grid size indicated in brackets): a stepparian flat plain (55 SU, a 5 x 11 grid), the rocky slope of a valley (16 SU, 4 x 4 grid), a grassland in the bottom of the valley (24 SU, 6 x 4 grid) and the central "mallín" of the valley (12 SU, 3 x 4 grid).

To determine the minimum sample size and the optimal shape and size of the SU, we analyzed the distribution pattern of the pellets in each habitat type, based on the pellets of the second count. The counting time of each type of SU was also measured.

We used the computer program *Ajuste* (developed in Fortran by J. E. Rabinovich) to test if the distribution of the pellets fitted the following discrete models: Poisson, negative binomial, Neyman A, uniform, Thomas, and Polya-Eggenberger. We verified the results obtained with *Ajuste* using the computer programs Poisson and Negbinom (Ludwig and Reynolds 1988). We tested the goodness of fit by a *G* test (Sokal and Rohlf 1981).

In cases where pellet distribution fitted a negative binomial model ($P > 0.05$) (Table 1), we used the following equation, derived from Gerard and Berthet (1971), to calculate the smallest sample size necessary to obtain an expected relative imprecision (coefficient of variation) in the mean number of pellets per unit area:

$$n = \frac{1}{CV^2 A} \left(\frac{1}{\bar{X}} + \frac{A}{k}\right) \tag{2}$$

where n = minimum number of SU, CV = expected coefficient of variation, A = area of the SU, $\bar{X}$ = mean number of pellets per SU, k = parameter of the negative binomial distribution model. The values of A, $\bar{X}$, and k were those corresponding to the samples obtained from each habitat and from the 0.5- and 1-m circles, as well as from the area delimited between both circles (called "ring" in Table 1).

In cases where the mean number of pellets per unit area (h) fitted the negative binomial distribution, the variance of the population was estimated through the equation given by Gerard and Berthet (1971):

$$Var\ (h) = \frac{h^2}{n\ A} \left(\frac{1}{h} + \frac{A}{k}\right) \tag{3}$$

where n = number of SU, A = area of each SU, k = parameter of the negative binomial distribution model.

The optimization of the sampling design was repeated during the following winter, to obtain a minimum sample size for this season. The same habitats were sampled using the number of SU derived from the optimization performed with summer data, plus approximately ten percent. The SU were established using the same design as that employed in summer. When winter data fitted a negative binomial distribution, the minimum sample size needed to achieve a 20% *CV* was calculated. We also estimated the variances and minimum sample sizes (for a 20% *CV*) for summer and winter data assuming a random distribution (Cochran 1981, page 110) to illustrate the bias that this assumption would introduce.

During the winters of 1989, 1990, and 1991, the sample size obtained for the 1988 winter season plus 20% (30 SU) was used to estimate hare densities in the central mallines of La Rinconada and the other six ranches. The effort was concentrated on sampling the mallines because previous studies (Amaya 1978, Amaya and Alsina 1982, Novaro 1991) and our pilot study indicated that hare activity was always higher in this habitat. To determine if the sampling scheme was robust to a large degree of variability in hare densities, we evaluated whether (1) the data from each ranch and year fitted the negative binomial model and (2) the coefficients of variation remained within the expected range.

The grids used in the pilot study were representative of a small portion of each habitat. Therefore, during 1989, 1990, and 1991 we distributed the SU in five grids of six (2 x 3) SU spaced every 400 m along each mallín. To establish if this change in sampling design would alter the mean and variance of the pellet counts, during the first winter (1989) we set both sampling schemes (grid and series of six SU) in four of the ranches. We compared the means and variances of the number of pellets with both sampling schemes in each ranch using the Wilcoxon matched-pairs signed-ranks test (Daniel 1978). As no significant differences were detected between the two designs (P = 0.25 for means and P= 0.875 for variances), during 1990 and 1991 we used only the series of six SUs to sample each mallin.

Finally, the total number of hares present in the mallines of each ranch was estimated by multiplying the hare densities in the mallines by the estimates of the area covered by mallin habitats in each ranch. These area estimates were obtained from the landowners, and ranged from 440 to 1,400 ha. A minimum estimate of the overall hare density in each ranch was obtained by dividing the number of hares in the mallines by the total area of the ranches. This minimum density could be useful for management purposes, as harvesting quotas must be determined for areas that are at least the size of a ranch (8,000 to 28,000 ha in the study area) (A. del Valle pers. comm.).

Results

No single habitat or design of SU produced data fitting the Poisson distribution model. Therefore, a random distribution of pellets cannot be assumed to estimate minimum sample sizes and variances. Similarly, no data set fitted the Neyman A, uniform, Thomas, or Polya-Eggenberger models.

Data from the plain, valley slope, and grassland sampled in summer, when the SU used was the 0.5-m circle, fitted the negative binomial distribution model (Table 1). For these habitat types, minimum sample sizes estimated ranged from 19 to 33 (Table 2). Data from the four habitats sampled in winter also fitted a negative binomial distribution model; minimum sample sizes estimated ranged from 20 for the valley grassland to 99 for the valley slope (Table 2).

Table 1: Fitting the frequency distribution of the number of European hare pellets per sampling unit (SU) to the negative binomial distribution model, for different types of SU and different habitats in southern Neuquen. NB (negative binomial) fitting indicates non-significant difference with the NB model (P= 0.05); k is a NB distribution parameter.

Habitat type	Type of SU	NB fitting	$\bar{X}$ pellets per SU	k
Plain	1 m	no		
	0.5 m	yes	3.01	1.25
	ring	no		
Valley slope	1 m	no		
	0.5 m	yes	4.93	1.92
	ring	no		
Valley grassland	1 m	no		
	0.5 m	yes	14.01	0.83
	ring	no		
Valley "mallín"	1 m	no		
	0.5 m	no		
	ring	no		

Table 2. European hare density and sample size in pilot study (n) for different habitats in La Rinconada, Neuquén, during the summer and winter of 1988; CVs and minimum sample sizes (Min. n) needed to achieve a 20% expected CV, according to the negative binomial (equation 2) and random distribution models.

Habitat type	n	Density (hares/ha)	Neg. binomial		Random	
			CV	Min. n	CV	Min. n
Summer						
Plain	55	4.6	0.15	31	1.09	30
Valley slope	16	7.6	0.22	19	0.87	19
Valley grassland	24	18.6	0.27	33	0.99	25
Valley "mallín"	12	27.9	*	*	0.46	8
Winter						
Plain	36	1.2	0.31	96	1.88	88
Valley slope	35	0.8	0.31	99	1.87	88
Valley grassland	35	4.7	0.15	20	0.86	19
Valley "mallín"	14	7.8	0.25	24	0.47	6

* did not fit the NB model

When a random distribution was assumed, the *CVs* for each habitat were two to seven times higher than when the equation for a negative binomial distribution was used (Table 2). The minimum sample sizes estimated assuming a random distribution were smaller in most cases than those obtained using the equation for a negative binomial distribution (Table 2).

The time needed to count the pellets in each SU type (the greater and smaller circles and the "ring") was 2.6 (SD = 0.7), 0.9 (SD = 0.2), and 1.7 (SD = 0.7) min, respectively. That means that counting pellets in 0.5-m circles takes approximately one-third the time taken for 1-m circles, and half the time of counting them in the ring delimited by both circles. However, time consumed by each SU type was not considered in the optimization, because only data from the smaller circles fitted a known distribution model, thus permitting estimation of a minimum sample size.

Pellet counts from 16 out of 17 series of 6-SU-grids established in each mallín during 1989, 1990, and 1991 fitted the negative binomial distribution model (Table 3), and none of them fitted the Poisson distribution model. The coefficients of variation of 12 series of grids were 21% or smaller, whereas the remaining four were 25% or larger (Table 3).

During summer of 1988 hare densities in all habitat types were approximately four times higher than during winter (Table 2). Densities in the mallines were higher than in the other habitats during both seasons (Table 2). Overall minimum density in each ranch ranged from 0.2 to 2.2 hares/ha in 1989, 0.1 to 1.4 in 1990, and 0.2 to 1.0 in 1991 (Table 3).

Discussion

Other methods employed to estimate densities of lagomorphs or similar mammals include spotlight transect, capture-recapture and removal (Dietrich 1984, 1985, Humphrey 1989, Lefebvre et al. 1982, Litavaitis et al. 1985, Smith and Nydegger 1985). Although these methods are better for estimating densities of lagomorphs than the indirect pellet-count method (Smith and Nydegger 1985), limitations of environmental conditions, cost, and manpower often preclude their use. The spotlight-transect

Table 3. European hare density (D), % CVs, and fit to the negative binomial distribution (*= fit; **= no fit; P= 0.05) in mallín habitats in Patagonia during the winters of 1989, 1990 and 1991. The figure between parentheses is the minimum density in each ranch, estimated from the number of hares present in the mallines and the total area of each ranch.

	1989		1990		1991	
Ranch	D	CV	D	CV	D	CV
La Rinconada	6.9 (0.4)	20*	1.8 (0.1)	42*	8.5 (0.5)	19*
Collun-Co	26.1 (1.3)	19*	--	--	14.9 (0.7)	16*
C. Pinos	43.4 (2.2)	21*	--	--	19.6 (1.0)	16*
Aquinco	13.0 (0.6)	17*	--	--	--	--
La Papay	45.6 (2.3)	17*	28.0 (1.4)	**	8.4 (0.4)	16*
Los Remolinos	26.2 (1.3)	19*	9.7 (0.5)	25*	7.3 (0.4)	17*
Catan Lil	4.9 (0.2)	33*	2.1 (0.1)	21*	3.7 (0.2)	29*

method requires availability of roads, which are usually lacking in the mallines. As the mallines are important hare habitat, the spotlight-transect method is not applicable in some areas of Patagonia. Capture-recapture and removal methods require a high trapping effort in each area to be sampled. Thus, they are inappropriate for sampling large areas to estimate population densities for management purposes. When the pellet-count method is the best alternative available, a special effort should be devoted to optimizing its design and implementation.

No previous studies using the pellet-count method have analyzed the pattern of spatial distribution of pellets in the estimation of animal densities. Flux (1967) pointed out that the distribution of European hare pellets is not random and our findings confirm a lack of fit to the Poisson distribution. In our study, the spatial distribution of pellets of European hares fitted an aggregated, negative binomial distribution model. Thus, the variance and minimum required sample size should be estimated based on this type of spatial distribution. When we estimated variance and sample size presuming a random distribution, much larger variances and somewhat smaller minimum sample sizes were produced. This illustrates the bias that the assumption of random distribution has introduced in previous studies. We suggest that when a pellet count or a similar sampling method is used, researchers should test different sizes and shapes of sampling units in a pilot study to determine in which case the data fit a known distribution model. If the data fit the negative binomial model, the equations for minimum sample size and variance provided in this study should be used.

To apply the optimization method suggested here, the habitat of the animal whose density is estimated must be relatively homogeneous (Gerard and Berthet 1971). We found differences in variances and sample sizes required between seasons and among habitats within our study area. For this reason we suggest that in future studies sampling schemes should be optimized separately for each habitat and season whenever animal densities fluctuate markedly during the year.

The density estimate in the pellet-count method is affected by variability in the defecation rate as well as in the number of pellets per SU. In this study we used the European hare defecation rate calculated in a different habitat (New Zealand; Flux, 1967) and with no estimate of its variability. In lagomorphs it has been reported that the type of food consumed affects the number of pellets produced (Arnold and Reynolds 1943). Therefore, for future studies we recommend that the mean and variability of the defecation rate of the study animal be estimated in the habitat where the sampling will be conducted.

In conclusion, we presented the optimization of a sampling scheme for the pellet-count method for European hares in Patagonia, based on the spatial distribution pattern of the pellets. We recommend that this distribution pattern be evaluated every time the pellet-count or a similar method is used to study animal densities or habitat use.

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