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Methodological problems related to spotlight count as a census technique for *Lepus europaeus* in an alpine environment.

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Abstract Population estimations are necessary for effective conservation management. In Italy, brown hare populations are commonly censused by spotlighting, but this method does not seem to suit an alpine environment due to its vegetational and orographical complexity. The aim of this study is to evaluate the critical aspects related to spotlight census method in an alpine environment. Spotlight was carried out along two transects of a typical alpine environment. Observed animals were used to define density (number of animal seen/100 ha) and method precision (coefficient of variation (CV) applied to monthly repeated observations). Animal detectability was evaluated using half-normal function with cosine expansion (Distance 5.0®). Animal observability was evaluated by analyzing density estimates related to habitat conformation (unseen areas or full visibility). The exact surface surveyed by spotlighting was evaluated, defining the observation spotlight beam range (OTA) and the land useful sighting (LUS). In the end, LUS was classified in three patches according to hare presence: no hares, occasional hare presence, and constant hare presence. To evaluate habitat influence onto CV, we used a bootstrap simulation. The results show that spotlighting alone is not the most suitable method to apply in the alpine environment because habitat structure highly influences census results. Recommendations to improve spotlight surveys for monitoring European brown hare populations are given.

Keywords Alpine environment. European brown hare . *Lepus europaeus* . Spotlighing

Introduction

Assessing and monitoring animal populations precisely and accurately are essential for research, management, and conservation (Caughley and Sinclair 1994; Wilson and Delahay 200 l). European brown hare populations seem to decline in the Italian alps (Gobbi et al. 2001; De Battisti et al. 2002) mainly due to the disappearing of agricultural activities and to landscape transformation with increase of uncultivated areas and woodlands. Such situation has led to the fragmentation of hare habitat in suitab1e insular patches not always connected to each other. Heterogeneity generated by fragmentation can create barriers to movement (Arnold et al. 1993) and, ultimately, lead to the local extinction of the species (Burkley 1989; Soulè et al. 1992). In this context, it is of paramount importance to use accurate and precise census techniques and strict monitoring plans.

During the past 50 years, a great deal of literature on methods for estimating population density and demographic parameters has been published. Methods of censusing have become more refined and sophisticated, and they should be individually designed or adapted to species behavior and to study aims (Sutherland 1996; Krebs 1999; Southwood and Henderson 2000). An extensive review of methods used to census lagomorph populations is presented in Langbein et all (1999). It includes indirect methods which rely on censusing signs of animal presence, e.g., faecal pellet count and track count, and direct methods which imply the possibility of seeing animals, e.g., clearance drives or total capture, belt assessment, drag line counts, spotlighting, vantage point counts, and markresight. Due to hares' nocturnal activity, one of the most used direct method is the spotlight count along line transect (Eltringham and Flux 1971*;* Frylestam 1981; Barnes and Tapper 1985). Spotlighting is also the main method used in lta1y to census European brown hare *Lepus europaeus,* both in the plain and in the mountain and hill habitats (Trocchi and Riga 2005). It assumes that it is possible to observe animals in alI directions and that the view should not be obstructed by obstacles. Very often, these assumptions are not met, and the validity of the data collected in the field has been questioned (Mahon et al. 1998; Sharp et al. 2001). Major concerns are (l) low precision (Edwards et al. 2000), (2) the species detection bias (Jones and Coman 1982; Mahon et al. 1998), and (3) habitat influence (Langbein et al. 1999). The observation of hares is not a problem in the plains, characterized by open habitats (Spagnesi and Trocchi 1993), but it is more difficult in the mountains and hills due to (1) a complex topography and (2) progressive loss of open areas caused by abandonment of traditional agriculture. This study evaluates the critical aspects related to spotlight census method in the alpine environment. In particular, we want to assess the validity of spotlighting techniques for detecting the abundance of brown hares by repeating spotlighting transects and calculating density estimates and associated levels of precision. We examine the consequences of two variability factors: detectability (probability of detecting a hare in the spotlight range) and observability (effects of nonvisib1e areas on density estimates). Final1y, we evaluate the precision of the method considering the coefficient of variation (CV). We discuss reasons for our findings and make recommendations for future spotlight surveys.

Materials and methods

Study areas

Spotlight counts were carried out in two study areas located in the Western Alps in Cuneo Province (Varaita VaIIey, Piedmont region; 44°58'N, 7°22'E; 900-1,100 m a.s.i.). The valley has a glaciofluvial origin with a U-shaped conformation, and maximum precipitations occur during spring and autumn. The first area (SAI) presents a southern exposition and is part of a protected area devoted for game reproduction. It is characterized by pastures and mowed meadows, woods, and scrublands; open areas are set amidst wooded formations *(Quercus petraea, Fagus sylvatica, TiZia sylvestris, Fraxinus excelsior, Castanea sativa,* and *Rosa canina).* The second area (SAl) has the same topography and vegetation, but hunting is allowed. Both areas are characterized by a mosaic of farmland and woodland areas, representative of the alpine environment; they present a cutting meadow regime with two harvesting periods (June-July and August-September), followed by a period of cattle pasture.

Sampling design

A transect of 4.2 km and a transect of 14.6 km were selected, respectively, in SAI and SA2 from the existing road network to sample the areas in a representative manner with respect to the available land use type. In SAI, the study was undertaken from September to April between 2003 and 2006; in SA2, during April between 2005 and 2009. We carried out three observation sessions in each month, also in case of snow cover, to test the precision of the method. Snow cover does not affect the census method. Since this method is high1y affected by changes in visibility, foggy and misty conditions were avoided. The theory and assumptions of spotlight count for hares and practical aspects of survey design are described by Eltringham and Flux (1971), Frylestam (1981), and Barnes and Tapper (l985). In this study, a4x4 car was driven along a transect, at low speed (15-20 km/h). Hares were searched for by two observers using a one million candlepower handhe1d spotlight. One observer swept the spotlight on both sides of the road, in front and behind the vehicle, and the other one scanned each lighted field for hares. The number of animals seen, the distance from the observer measured perpendicular to the route using a laser range finder (Leica LRF 900 scan), the distance travelled (kilometers from the beginning of the transect), and the GPS localization were recorded and reported in a CTR (Region Technica1 Map, ltaly) in scale of 1:10,000 with ArcView v3.3 GIS software (ESRI, Readlands, USA). The transects were surveyed from 2 h after sunset.

To evaluate the detectability of animals at different distances from the observer, we used half-norma1 function with cosine expansion as the se1ected model (Distance 5.0®). The software use the "distance sampling approach" based on the assumption that animal detectability is related to their distance e from the observer (detection function). The detection function allows for correction for the missing individuals (Buckland et al. LVV 1).

To evaluate the observability of hare spotlight count in mountain habitat, we analyzed density estimates related to habitat structure, considering unseen areas or assuming full visibility. Density estimates were ca1cu1ated as the number of animals seen/100 ha. We used geographic information systems (ArcView 3.2®), land use maps 1:10,000, and digital elevation model with 50-m resolution (piedmont Region).

According to Barnes and Tapper *(1Yì\5)* and Pegel (l *Yì\6),* we calculated:

1. observation spotlight beam range: effective strip width in which hares could be detected; observation theoretical area applied to linear transect (OTAL): application of the hare observation range, as buffer, to hypothetic linear transects;
2. observation theoretical area (OTA): application of the hare observation range, as buffer, to the transects considering the bends of the roads;
3. Land useful sighting (LUS): utilization of land use maps, orthophotos (CGR, Parma, ltaly) and digital elevation model to subtract from OTA the zones c1assified as wood and urban area and the zones over which the view is obstructed (presence of barriers as woods, scrubs, buildings, undulating terrain; Pegel 1986). Using digital elevation model and the view shed function in the Spatial Analyst package (Esri-ArcView 3.2®), we exc1uded areas not observable due to their orography.

LUS was c1assified in three patch typologies according to the presence of detected hares: patch 0= LUS without detections (encounter rate = 0), patch l = LUS with an occasional detection (0 < encounter rate < 1), and patch 2 = LUS with animals constant1y detected during the study period (i.e., animals aggregated; encounter rate = l).

Precision was calculated using the coefficient of variation (Davis 1982) applied to monthly repeated observations (CV = standard deviation x 100/mean observed hares). The habitat influence on the CV and their relationship was modelized using bootstrap analyses (Sokal and Rohlf 1995) to identify a threshold area to improve precision. Random distribution of 10,000 observations was plotted in the study areas, and CV for each visible patch was calculated. Sampling intensity was determined arbitrarily at the point where CV reached an asymptote and did not increase marked1y with an increase in sample size (Greig-Smith 1957).

The Mann-Whitney *U* test was used to assess differences among patch areas. Data were reported as means; statistical significance was set at $p<0.05$. Statistical analyses were performed using R 2.6.0 software® (R Development Core Team 2007).

Results

Detectability

The observation spotlight beam range was evaluated during a preliminary sampling carried out in the same period and in the same areas of the present study during which 108 hares were observed by spotlighting. All these animals have been observed within a distance of 120 m from the observer (unpublished data). This distance was used to define a buffer along line transects in both study areas, obtaining OTA and LUS values (Table l and Fig. I). The 108 hares were also used to define the probability of observation at various distances (Fig. 2). Observation histograms fitted well the detection function (chi-square goodness of fit test ═ 14.3; *p=0.22).* Detection probability calculated on detection function was 33.4 %.

Observability and precision

SA1

During the study period, 194 hares were observed by spotlighting (Table 2). Rare density ranged from 1.8 to 12.3 animals/100 OTA ha (mean ═ 5.3) and from 7.5 to 51.9 animals/100 LUS ha (mean ═ 22.3). The highest density was recorded in November (mean ═ 36.8 animals/100 LUS ha) and the lowest in April (mean = 15.1 animals/100 LUS ha). CV ranged from O % in December 2006 to 109.0 % in February 2004 (mean = 43.9 %).

SA2

During the study period, 38 hares were observed by spotlighting (Table 2). The density per transect ranged from 0.1 to 1.5 animals/100 OTA ha (mean = 0.7) and from 0.4 to 6.2/100 LUS ha (mean 3.0). CV managed from O % in April 2006 to 173.2 % in April 2009 (mean = 63.7 %).

SA1 and SA2

The three patches identified in each study area presented different mean area dimensions: patch 0=0.59 ha, patch 1= 1.65 ha, and patch 2= Il.80 ha. Significant differences were recorded among areas of these patches (patch 0 vs. patch l: W = 228 and $p<0.001$; patch 0 vs. patch 2: W= 0 and p<0.001; patch l vs. patch 2: W = 0 and $p<0.001$. Patch 2 represented the 13.6 % of OTA and the 55.8 % of LUS.

Rare observations were more frequent and continuous in some areas than in others, determining the variability of CV values (SAI CV = 43.9 %; SA2 CV = 63.7 %), but excluding occasional detections from the survey, the CV decreased to 27.3 % (reduction of 37.8 %) in SAI and to 20.2 % (reduction of 68.3 %) in SA2. The model equation is $y=1.62^{x-O.71}$ (Fig. 3), and it suggests survey patch areas of about 9 ha to obtain acceptable CV

Discussion

Our results show that spotlighting, as described in the literature (Eltringham and Flux j 971; Frylestam 1981; Bames and Tapper 1985), is not the appropriate census method to apply in the alpine environment. The main problem is due to the alpine landscape in which hedge rows, undulating terrain, forest, buildings, and blind spot behind these often obstruct sight so that many hares can be missed, badly influencing the accuracy and precision of the method. Such habitat characteristics ("unseen") were very important in our study areas, involving 76.3 % of the environment in SAI and 75.5 % in SA2.

Hare densities obtained considering OTA or LUS are very different, showing that habitat structure highly influences census results, and therefore, that population density comparisons between areas with unknown habitat composition are questionable. To obviate this problem, we propose to define a "habitat standardization coefficient" which considers the LUS/OTA density ratio (in this study = 24 %) to normalize OTA density estimations. Another way to normalize data is to use the detection probability value (detectability standardization coefficient; in this study = 33.4 %). Indeed, the study of the habitat structure (LUS/OTA) and the study of detection probability are two methods that, starting from different assumptions, have similar conclusions (similar standardization coefficients). From an "on field" point of view, both methods are not so easy to apply because they require access to sophisticated geographical information systems. We suggest the use of the habitat standardization coefficient because it defines the OTA and LUS parameters for each transect only once and can be applied year by year without further calculations.

To obtain a satisfactory precision is apparently a problem in the alpine terrain, and this was visible in high CV values caused by high habitat fragmentation. Applying a bootstrap simulation, we defined a threshold patch area over which the census method presents high precision. Such finding is confirmed by our data: considering patch 2, we obtained a CV of 27.3 % (with reduction of 37.8 %) in study area l and a CV of 20.2 % (with reduction of 68.3 %) in study area 2. These CV values (<22.5 %) are in accordance with the results of Marchandeau and Gaudin's study (1994) and are acceptable both for a hunting area characterized by low animal density and for a protected area characterized by medium-high density. Apparently, the CV reduction is due to an increase of the ratio perimeter/area of the patches: the smaller the surveyed patch area, the more casual is the possibility to observe an animal (in open areas of more than 9 ha, the probability to census a hare is statistically higher).

In conclusion, spotlight count is a simple method to obtain information of hare densities, but the interpretation of the data is highly complex and could lead to erroneous conclusions (Wincentz Jensen 2009). To achieve valid estimates in the alpine environment, a correction of the raw data is necessary. We, therefore, propose two types of counts: (l) point transect spotlight count in preselected open areas of at least 9 ha and (2) line transect spotlight count associated to distance sampling method which allows for correction for the missed individuals (Buckland et al. 200 l).

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Fig. 1 Observation theoretical area (OTA) and land useful sighting (LUS) in study area 1.

Fig. 2 Number of hare sightings (about 8-m interval grouping; *blue columns)* at different distances from the observer using the spotlight census method and Distance 5.0®, where good model fit was obtained (detection function is uniform key with cosine expansion; *red line)*. Sample size = 108 hares.

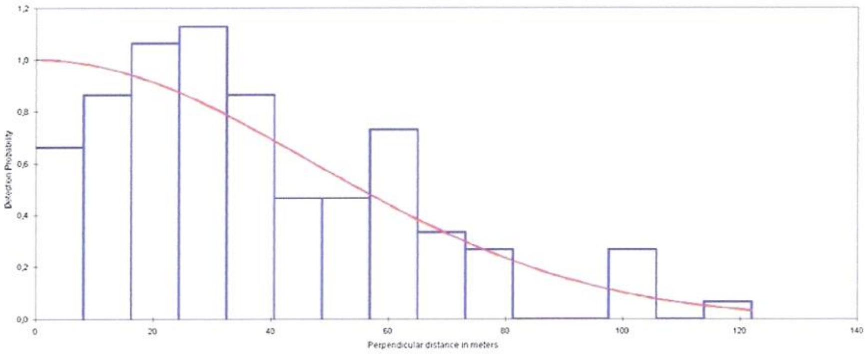

Fig. 3 Bootstrap analysis for the determination of threshold patch areas: relationship between dimension of open areas (ha) and CV (%).

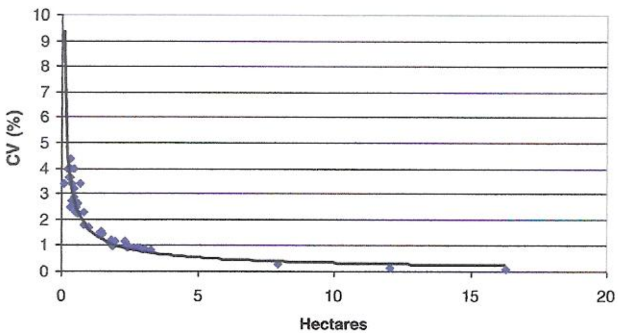

Table 1 OTAL, OTA (observation theoretical area), land use characteristics, and LUS values for each study area.

Study area	OTAL (ha)	OTA (ha)	Land use characteristics		LUS (ha)	Total length of transect (km)
			Presence of wood and urban buildings (%)	Obstructed view (%)		
1	105.3	84.1	62.8	13.5	19.9	4.2
2	354.9	350.5	62.4	13.1	85.8	14.6

OTAL observation theoretical area applied to linear transect, *OTA* observation theoretical area, *LUS* land useful sighting

Table 2 Results of spotlight counts for each study area and for each study period.

Study area	Total number of hares seen	CV (%)	Theoretical density (OTAL)	Exact density (LUS)
1	194	43.9	5.27	22.27
2	38	63.7	0.7	3.0

CV coefficient of variation