Allegato 2b_1

The use of block counts, mark-resight and distance sampling to estimate population size of a mountain-dwelling ungulate

Luca Corlatti [1, 2], Lorenzo Fattorini [3], Luca Nelli [4]

[1] Institute of Wildlife Biology and Game Management, University of Natural Resources and Life Sciences Vienna, Gregor-Mendel Strasse 33, A-1180 Vienna, Austria.

[2] Alpine Wildlife Research Center, Gran Paradiso National Park, Via della Rocca 47, 10123 Torino, Italy

[3] Department of Economics and Statistics, University of Siena, Piazza S. Francesco 8, 53100 Siena, Italy.

[4] Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy.

Corresponding author: Luca Corlatti
E-mail address: luca.corlatti@boku.ac.at

Abstract

Population size estimates represent indispensable tools for many research programs and for conservation or management issues. Mountain ungulates in open areas are often surveyed through ground counts that normally underestimate population size. While the use of sample counts is desirable, few studies have compared different probabilistic approaches to estimate population size in this *taxon*. We compare the size estimates of a male population of Alpine chamois using mark-resight and line transect sampling methods, while block counts were used to obtain the minimum number of males alive in the study area. Surveys were conducted within the Gran Paradiso National Park (Italy), in August-September 2013, using block counts along purposely selected paths and vantage points, mark-resight over 5 consecutive resightings from vantage points and paths, and line transect sampling along 12 transects repeated 8 times. Block counts yielded a minimum number of males alive in the population of N=72 individuals. This value was greater than the upper bound of the 95% confidence interval achieved using line transect sampling (N=54, SE=14%, 95% CI: 40-71) while mark-resight yielded a more realistic result of N=93 individuals (SE=18%, 95% CI: 63-137). Our results suggest that line transect sampling performed poorly in the Alpine environment, leading to underestimates of population size, likely due to violations of some assumptions imposed by the rugged nature of the terrain. The mark-resight yielded lower precision, possibly due to the limited number of marked individuals and resighting occasions, but it provides robustness and accurate estimates when marks are evenly distributed among animals.

Introduction

Population size estimates may represent effective tools in support of many research programs and for conservation or management issues. Researchers, conservationists and wildlife managers may be interested in obtaining accurate estimates of population size to investigate, for example, if a population is too small, i.e. endangered, too large, e.g. threshold levels in wildlife disease systems (Lloyd-Smith et al. 2005), or to explore the relationship between density and life-history traits (e.g. Stephens et al. 1999). Contextually, different stakeholders may be interested in the variation of population size over time, for example to explore its responsiveness to changes of environmental factors and to different management regimes (Yoccoz et al. 2001). In this latter case, accurate and precise estimates may not be necessary, provided that reliable population indexes are available (Morellet et al. 2007).

Although a multitude of statistical methods for estimating abundance has been proposed (Seber 1982; Schwarz and Seber 1999; Buckland et al. 2000; Pollock et al. 2002), single yearly ground counts such as the Block Counts (BC) remain among the most widely used methods to evaluate the size of mountain ungulate populations living in open areas above the timberline, where high visibility is ascertained (Largo et al. 2008). Ideally, to assess the actual population size, this approach would require perfect detection and animals immobile with respect to the observer (Lettink and Armstrong 2003). The difficulty to meet these assumptions, however, often leads ground counts to underestimate population size, as shown in Alpine ibex *Capra ibex* (Gaillard et al. 2003) or in Alpine chamois *Rupicapra r. rupicapra* (Loison et al. 2006). This, in turn would make ground counts unsuitable for attaining accurate size estimates. Nonetheless, ground counts have been widely used in guise of indexes of population abundance to investigate population dynamics (Jacobson et al. 2004; Willisch et al. 2013) or for planning management actions (Sinclair et al. 2006).

When the aim is to obtain statistically sound estimates of population size, however, the use of methods that take into account non-perfect detection is desirable. To this end, mark-resight (M-R) (Schwarz and Seber 1999) and distance sampling (DS) (Buckland et al. 2001) stand among the most widely used methods to estimate population abundance.

The basic idea behind M-R is that a sample of animals from a population is captured, marked and released. Further on a survey is conducted. In its simplest form, the M-R method assumes that the proportion of marked animals observed (rather than recaptured) in this survey is an estimate of the unknown proportion in the whole population. Usually, however, reliable estimates of population size can be obtained conducting several surveys after marking (Otis et al. 1978). M-R methods may imply great costs especially in terms of capturing effort, but if a good proportion of marked animals is available and a sufficient number of resightings is conducted, good estimates can be obtained, provided that the underlying assumptions adopted to construct the M-R estimators are met. M-R methods, tested with a known population of mule deer *Odocoileus hemionus* (Bartmann et al. 1987), have been applied to investigate the population size of several *taxa* including ungulates (e.g. white-tailed deer *Odocoileus virginianus*: Rice and Harder 1977), bears (Miller et al. 1987), canids (Hein and Andelt 1995) and phocids (e.g. harbour seals *Phoca vitulina*: Ries et al. 1998). Furthermore, M-R has found wide application in the size estimate of mountain ungulates such as the mountain sheep *Ovis canadensis* (Furlow et al. 1981; Neal et al. 1993), Alpine ibex (Largo et al. 2008) and Alpine chamois (Loison et al. 2006; Fattorini et al. 2007a).

When marked animals are not available, DS may represent an alternative for estimating population size. DS is an extension of plot sampling: an issue of the plot sampling method is that the size of the area used to calculate the density is fixed *a priori* and it is assumed that all the objects within that area are detected (Buckland et al. 2001). In DS, the assumption that all animals are counted is relaxed, and the probability of detection is estimated from the distances to the observed animals (Buckland et al. 2001). This method is relatively easy to perform; it requires neither marked animals nor the mandatory count of all the animals in the surveyed area, and a few observers can conduct the observations. DS methods, however, require a great number of individuals, or clusters of individuals, to observe (> 80), and rather stringent underlying assumptions (Buckland et al. 2001). The most familiar version of distance sampling (known as conventional distance sampling or CDS) crucially assumes that: a) transects are placed randomly onto the study area; b) the probability of observing any animal only depends on its distance from the transect; c) detection probability is one for animals on the transect. In practice, CDS is based on the idea that all animals are equally approachable when walking the transects and each of them is characterised by the same detection probability, determined from an assumed detection function estimated from the observed distances. While a) is necessary to ensure that all the animals are equally approachable from the transects, a) and b) jointly ensure that any animal has the same detection probability; c) allows the estimation of this probability from the observed distances. As pointed out by Buckland et al. (2004, Section 10.3), CDS modeling is kept to a minimum and the resulting estimator is far more defensible than other DS estimators, which require assumptions on the distribution of animals over the study area. Other less crucial assumptions of DS usually require that: d) distances are measured accurately and e) animals do not move toward or away from observers before detection. While d) does not represent a veritable assumption, since it can be ensured by proper training and technology, e) is obviously unrealistic but it can be handled at the estimation level by introducing constraints on the detection function (Thomas et al. 2010). DS has been widely applied to various *taxa* as diverse as cetaceans (Barlow 2006), birds (Bächler and Liechti 2007) and primates (Buckland et al. 2010) and also to ant nests (Baccaro and Ferraz 2012), but its application in mountainous environments remains limited (Pérez et al. 2002; Newey et al. 2003) possibly owing to the difficulties in travelling randomly selected linear transects over rugged terrains.

A number of studies have proposed pairwise comparisons of population estimates using different methods, including the ones described above, in mountain-dwelling species, such as ground counts and M-R in Alpine chamois and Alpine ibex (Loison et al. 2006; Largo et al. 2008), spotlight counts and M-R in red deer *Cervus elaphus* (Garel et al. 2010), BC and DS in Southern chamois (Herrero et al. 2011), or plot sampling and DS in black grouse *Tetrao tetrix* and rock ptarmigan *Lagopus muta* (Franceschi et al. 2014). Yet, to our knowledge, very few studies have so far concurrently compared ground counts, M-R and DS in mountain ungulates (e.g. García-González et al. 1992 on Southern chamois; Wingard et al. 2011 on argali *Ovis ammon*), possibly due to the challenges posed by the rugged nature of the mountainous terrains – either in terms of capturing or monitoring effort.

The chamois, Genus *Rupicapra*, is a weakly dimorphic mountain ungulate, excellently adapted to live in rugged, rocky terrains. The chamois is the most abundant mountain ungulate of Europe and the Near East; populations of the Alpine subspecies can be found also in the mountain range of New Zealand as a result of past translocations (Corlatti et al. 2011). Female chamois and their kids live in herds, while adult males tend to live solitary for most of the year (Krämer 1969); during the warm period, chamois tend to use open areas at high elevations (Lovari et al. 2006), characterised by good visibility. As a consequence, it has been traditionally assumed that chamois population size can be easily assessed through ground counts performed once (spring or summer) or twice (spring or summer and fall) a year (Houssin et al. 1994) and very few attempts have been made to estimate chamois population size using probabilistic sampling approaches such as M-R (Loison et al. 2006; Fattorini et al. 2007a) or DS (Herrero et al. 2011). Although the chamois, as a Genus, is not threatened (*sensu* IUCN), some *Rupicapra* subspecies are threatened

(Corlatti et al. 2011). Furthermore, the chamois represents an important game animal throughout large part of its distribution range. The use of effective tools to estimate population size may therefore be of great importance for conservation and management purposes.

Taking advantage of a sample of marked individuals, in this work we investigated the size estimates of a protected male population of Alpine chamois using M-R and DS; we used BC only to obtain the minimum number of males alive in the study area. Our analysis aims to provide useful information on the relative performance of methods that can be used in the monitoring of *Rupicapra* populations and, more generally, of mountain-dwelling ungulates.

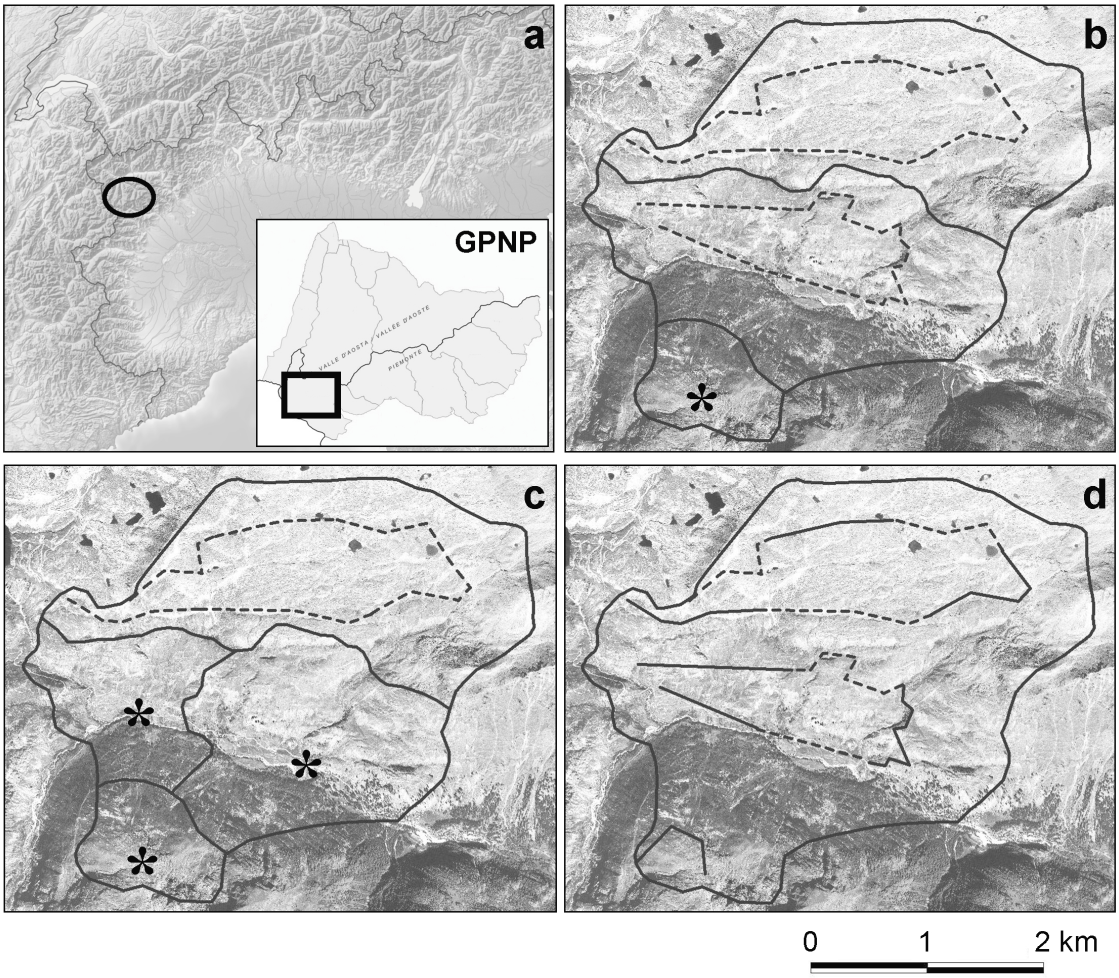
Materials and methods

Study area and population

The study area, whose boundaries were chosen *a priori* – following natural features such as ridges and valleys – extends over 10 km^2 between 1,800 and 3,000 m a.s.l in the upper Orco Valley (Gran Paradiso National Park –GPNP–, Western Italian Alps, 45°26’30” N, 7°08’30” E) (Fig. 1a). The continental climate, with mean yearly rainfalls of about 1,096 mm, shows mean temperatures between -3.7 °C in winter and 13.1 °C in summer (La Morgia and Bassano 2009). The valley is oriented west to east, with a south-facing slope –that represents ca. 90% of the study area– dominated by meadows of coloured fescue *Festuca varia*, and a north-facing slope where woods of larch *Larix decidua* and patches of alder shrubs *Alnus viridis* are present. The chamois population of the GPNP has not been hunted since 1922, and the main limiting factor is likely winter starvation, whose severity may change according to sex, age and winter harshness (Rughetti et al. 2011). Other ungulate species present in the study site are Alpine ibex and roe deer *Capreolus capreolus*.

Between February 2010 and December 2012, thirty adult male chamois (i.e. ≥ 2 years of age) were darted by the personnel of the Park with a mixture of xylazine and ketamine. We estimated the age of each individual by counting horn notches (Schröder and von Elsner-Schack 1985), and we equipped all chamois with individually recognizable GSM-GPS Pro-Light collars with VHF beacon device (Vectronic Aerospace GmbH, Berlin) and ear tags. Nussberger and Ingold (2006) showed that the behaviour of Alpine chamois is not affected by radio-collars. The sedative effect was reversed by an injection of atipamezole (Dematteis et al. 2009). The entire procedure usually took less than 40 minutes and was always performed with the assistance of a veterinarian. These methods are in line with the Italian law. By the time of the surveys carried out in this work (August-September 2013), only a subset of GPS devices were still working, but the VHF data confirmed that N=14 individuals were present within the study site.

Figure 1. a) Location of the study site within the Gran Paradiso National Park; b) sectors, paths and vantage point (asterisk) used for the block count survey within the study site; c) sectors, path and vantage points (asterisks) used for the mark-resight survey conducted over 5 consecutive days within the study site; d) line-transects used for distance sampling survey within the study site (solid and dashed lines indicate different transects). The spatial scale refers to figures b), c) and d).



Block counts

Block counts were conducted on September 3, 2013 by the park wardens of the GPNP (3 observers), who surveyed the entire study area over 1 single day, within the framework of the annual, long-term numerical monitoring of ungulates carried out since 1956 in the Park (see Jacobson et al. 2004). The study area was subdivided into 3 sectors: each sector was surveyed by 1 observer, from 6 a.m. to 9 a.m, walking along paths or from vantage points purposely selected in order to guarantee the good visibility of the study area (Fig. 1b). Observations were conducted with the aid of proper optical instruments (binoculars Swarovski 10x40 and spotting scopes Swarovski 20-60x65). Every spotted animal was identified by age- and sex-class and georeferenced.

Mark-resight

Between September 21 and September 25, 2013, two observers (including one of the authors, LC) conducted 5 sessions of resighting, over 5 consecutive days. During each session, observer A surveyed 3 sectors – consecutively – from vantage points, while observer B surveyed 1 sector walking along purposely selected paths (Fig. 1c). For practical reasons, surveys took place between 4 p.m. and 8 p.m,, but male chamois do not show significant differences in activity rhythms between morning and afternoon (Corlatti and Bassano 2014). Observations were conducted with the aid of proper optical instruments (binoculars Zeiss and Swarovski 8/10x40 and a spotting scope Swarovski 20-60x65): all occurrences of marked and unmarked adult males (i.e. ≥ 2 years of age) were noted down, and every spotted animal was georeferenced (Table 1). During the surveys, every marked animal was uniquely identified, and the GPS and VHF data confirmed that the N=14 marked males were evenly distributed over the study area, and their number remained constant throughout the 5-days sampling. Since the counting procedure was conducted in a relatively short time, and the movements of marked animals suggested no evidence of emigration/immigration events from/into the study area, we adopted a closed-population assumption (Otis et al. 1978) to estimate population size using M-R method.

Several estimators are available for assessing closed-population abundance when the number of marked individuals is known: they include, among the others, the Joint Hypergeometric Maximum Likelihood Estimator (JHE) (Bartmann et al. 1987), the Minta–Mangel Estimator (MME) (Minta and Mangel 1989) and Bowden's Estimator (BOWE) (Bowden and Kufeld 1995), all implemented in program NOREMARK (White 1996). In their investigation of these estimators' robustness, Fattorini et al. (2007a) motivated the use of BOWE, which proved to be the sole reliable procedure for estimating population size, offering computational simplicity and robustness, provided that marks are quite evenly distributed among groups. BOWE relaxes several assumptions of M-R, allowing great flexibility: it does not require sampling without replacement within resighting occasions, homogeneous probability of resighting and independence among resightings of different animals. More recently, the Beta-Binomial Estimator (BBE: McClintock et al. 2006) and the Logit Normal Estimator (LNE: McClintock et al. 2009) have been proposed. LNE has been also implemented in the framework of program MARK (White and Burnham 1999). Even if BBE and LNE do not require all animals to have the same resighting probability within occasions, both of them ultimately require that the number of resightings of each animal constitutes a set of independent and identically distributed random variables. This assumption, however, proved unrealistic in the framework of M-R methods

(Fattorini et al. 2007a). We therefore conducted the analysis of data using BOWE implemented in NOREMARK.

A second – independent – M-R estimate, again using BOWE, was reckoned adopting the data collected during distance sampling (see below) (Table 1).

Table 1. Number of observations per individual marked male chamois, and number of unmarked male chamois observed during mark-resight sessions conducted in late September (5-session mark-resight) and between mid-August and mid-September (using distance sampling data collected over 8 sessions) in the study site within the Gran Paradiso National Park in 2013.

	Animal ID														
Survey	m4	m5	m8	m11	m12	m13	m17	m18	m24	m25	m26	m28	m31	m33	Unmarked
Mark-resight (5 sessions)	4	3	0	3	1	2	1	3	0	2	0	1	2	1	135
Mark-resight (distance data)	3	6	1	6	0	5	0	0	0	1	1	4	7	1	193

Distance sampling

Between August 17 and September 17, 2013, one of the author (LC) walked 12 transects within the study area; each transect, ca. 1.1 km long, was surveyed 8 times, for an overall of 96 occasions and 110 km walked over 34 days. Due to the rugged nature of the Alpine terrain, transects were mandatorily placed along pre-existing paths (Fig. 1d), thus violating one of the basic assumption of CDS. Transects were walked between 4 p.m. and 8 p.m., and given that 6 transects could be walked in one day, the study area could be surveyed over two consecutive days. Observations were conducted without the aid of binoculars, which were used solely to clarify group size, identify the sex and age of animals, and the occurrence of marked individuals. When adult males (i.e. ≥ 2 years of age), or groups of adult males (defined as animals within a radius of ca. 50 m) were seen, we measured their perpendicular distances from the line of the transect using a Svarowski laser rangefinder. Vertical angles were measured using a Suunto clinometer. If animals occurred in groups, the perpendicular distance from the transect line to the centroid of the group was measured. As seen above, the GPS and VHF data of marked animals suggested no evidence of emigration/immigration events from/into the study area during the surveys; this, together with the small home range sizes registered for marked male chamois in August-September (10-20 ha: Corlatti, unpublished data), in turn suggests that the numerical size of the male chamois population in the study area remained fairly stable during the survey period.

To analyse these data, we used the CDS engine of the program DISTANCE 6.0 (Thomas et al. 2010). Observed distances were pooled to obtain a global estimate of the detection function, expected cluster size and population size. We performed estimation starting from half-normal, hazard-rate and uniform key functions with Hermite and simple polynomial series adjustment terms. We chose the best model according to the Akaike's Information Criterion (AIC) and goodness-of-fit statistics. To estimate the expected cluster size we used both size-bias regression method and, if the regression was not significant, we averaged the size of detected clusters. The sampling variance was estimated empirically by nonparametric bootstrap, resampling from the 96 occasions.

Issues of double counting of animals, possibly due to chasing of individuals during surveys, were unlikely to occur over all the survey occasions: the chamois population in the GPNP has been protected since 1922 and animals are greatly habituated to the human presence. The success of chamois darting – which requires to approach animals up to at least 40-50 m – and the investigations conducted on the same population in recent years (e.g. Corlatti et al. 2013a, 2013b) – which required observation of animals at close distances – reinforce our assumption. Furthermore, chamois antipredatory behaviour typically relies upon the use of foraging areas near rocks (e.g. Chirichella et al. 2013) where animals can rapidly find refuge, when disturbed, without the need to cover large distances.

Results

Block counts

The Block Counts yielded a minimum number of adult males chamois alive in the population of *N*=72, in line with the figures registered for the study site in the recent years.

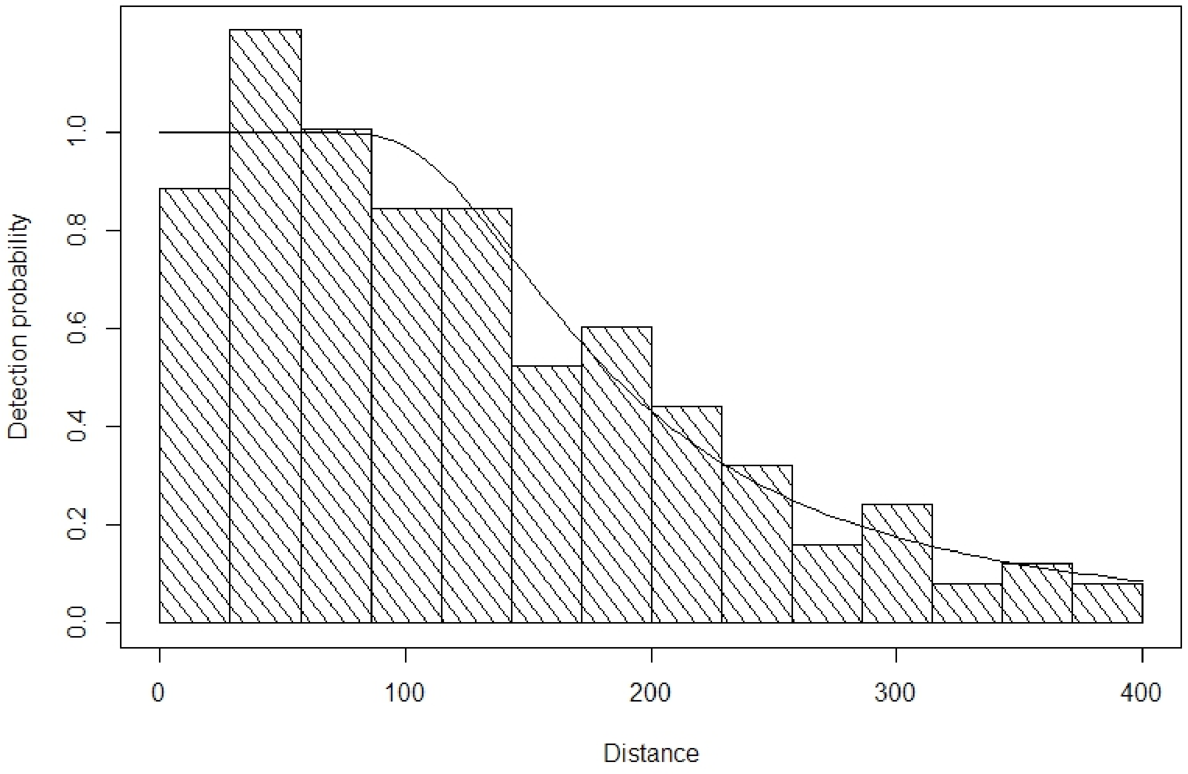
Mark-resight

The Bowden's method applied to the mark-resight survey, conducted over 5 consecutive sessions from vantage points and paths with the aid of optical instruments, yielded an estimate of *N*=93 adult males with an estimated SE of 18% (CI: 63-137), (see details in Bowden and Kufeld [1995] on how to estimate SE). When we applied BOWE to the data collected during distance sampling (8 resighting sessions along paths, without the aid of binoculars) we obtained similar results, but a slightly smaller precision leading a wider confidence interval: *N*=86, SE estimate of 24% (CI: 52-142). The difference of 7 animals between the two estimates assessed by means of the two-sided t-test was highly not significant (*P*=0.79)

Distance sampling

On the whole, we collected 187 observations for 230 adult males with a mean cluster size (±SD) of 1.23 (±0.043). The best detection function for adult males was a hazard-rate function (σ = 141.3 ± 15.95, β = 2.67 ± 0.405) without series-term adjustments (AIC = 2160.88, GOF K-S P = 0.883) (Fig. 2). The effective strip width was 193.11 m and the abundance estimate was N=53 males with an SE estimate of 14% (CI: 40-71).

Figure 2. Histogram of the perpendicular distance from transect to detected male chamois and detection function giving rise to the best fitting (in terms of AIC) obtained using the hazard-rate key function without series-term adjustments.

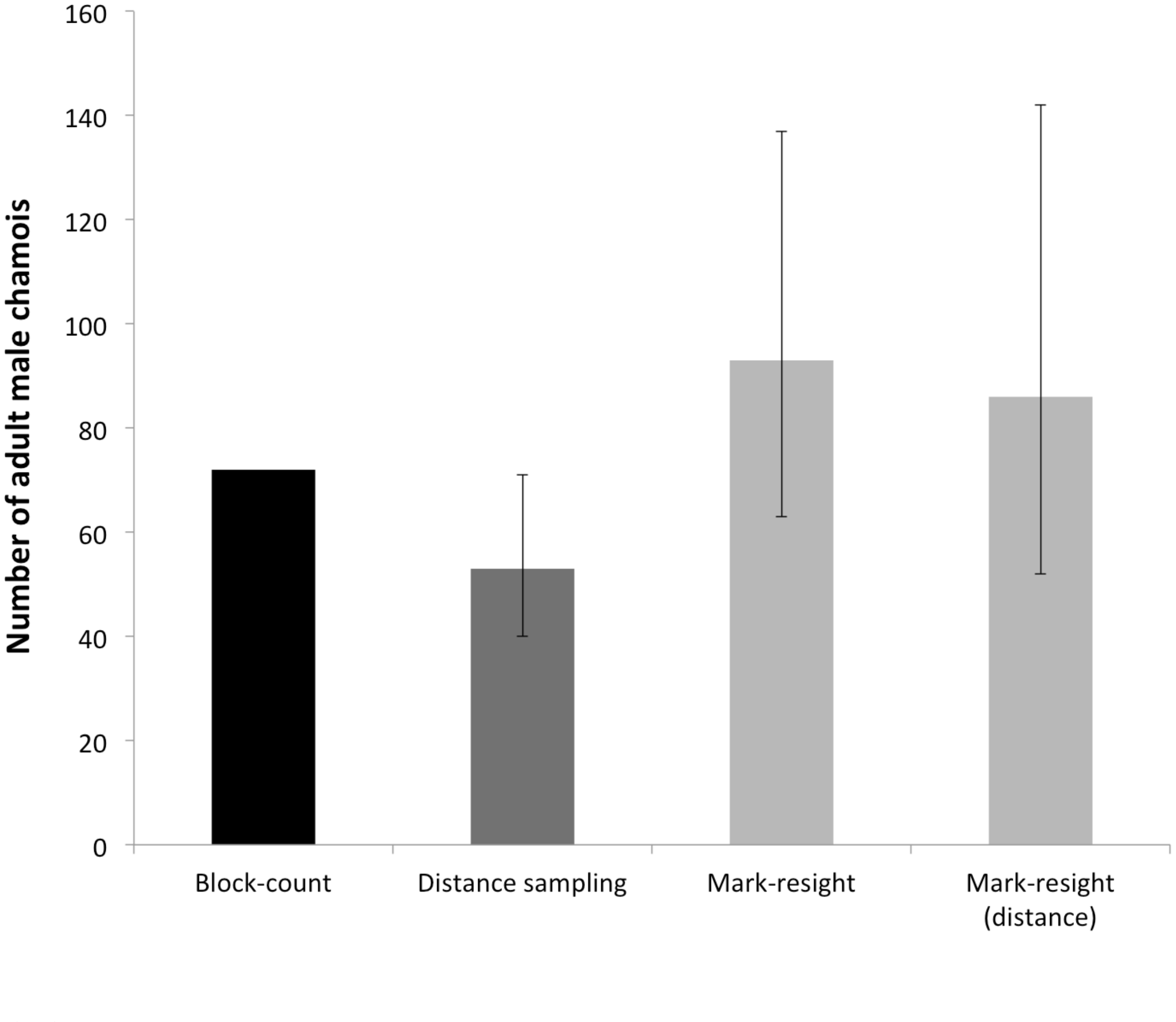


The difference of 40 animals between the BOWE estimate from the M-R experiment and the DS estimate, assessed by means of the one-sided t-test, proved the DS estimate significantly smaller than the BOWE estimate (P=0.015).

Discussion

Fig. 3 summarizes the results obtained using BC, DS and M-R. In the absence of an experimental setting, where the true population size is known, assessing which method yielded the best estimate is challenging. However, the minimum number of males alive obtained from BC provides some useful insights on the accuracy of DS and M-R estimators, while insights on their precision can be derived from the resulting SE estimates.

Figure 3. Size estimates of a male chamois population in the GPNP obtained using distance sampling and mark-resight between August-September 2013 compared with the minimum number of males alive achieved by means of block counts. The last rectangle refers to the estimate obtained applying the mark-resight method to the data collected during distance sampling.



Block counts

BC often leads to underestimates of population size in mountain ungulates (Loison et al. 2006). Gaillard et al. (2003), for example, showed that block counts consistently underestimated by at least 20% the numerical size of a population of the Alpine ibex, a mountain-dwelling ungulate that shares the summer habitat with Alpine chamois. Despite the good visibility offered by open areas, the rugged nature of the mountainous terrain, due to the presence of rocks, cliffs, ridges and valleys, may in fact restrict the ability to detect animals. Seasonal detectability rates (n. marked males observed / n. marked males present) calculated over the past years thanks the presence of chamois males marked with GPS-collars, supports this suggestion: 0.51 in spring (± 0.14 SD), 0.34 (± 0.14 SD) in summer, 0.35 (± 0.14 SD) in autumn and 0.64 (± 0.15 SD) in winter (Corlatti, unpublished data).

It remains unclear whether similar levels of bias may apply to the whole population, as well as to populations living in different areas: potential disparities in detectability due to sex- and age-effects, or to diverse morphological features, may lead to different levels of underestimations. Daily meteorological conditions may also strongly affect animals' activity and distribution (Aublet et al. 2009), possibly leading to different estimates over different sighting trials. Furthermore, Gaillard et al. (2003) pinpointed a saturation effect when comparing BC and M-R in an Alpine ibex population at intermediate and high densities, and further studies are needed to verify the absence of a saturation effect when using BC in Alpine chamois.

Consequently, our study did not aim to investigate the reliability of BC per se, but it rather aimed to assess the minimum number of chamois males alive in the study area, that could be later used to check the estimates of probabilistic methods.

Mark-resight

The goodness of M-R estimates mainly depends on the possibility to accurately meeting the underlying assumptions of the method. Estimates of closed populations rely upon 2 basic assumptions: a) there is no birth, immigration, death or emigration during the study and b) marks are not lost (Otis et al. 1978). The assumption of closure in our study area is supported by two considerations: none of the collared animals left the study site during surveys, and counts were conducted over a short time frame, which makes events of immigration and emigration (or death) unlikely. The second assumption, with the use of GPS-VHF collars, did not represent a problem. Each M-R estimator, in turn relies upon a set of specific assumptions: Bowden's estimator, in particular, assumes that a) the total number of resightings for each animal constitutes a set of fixed values and b) the animals to be marked are selected from the population by means of simple random sampling without replacement. Even if both assumptions proved unrealistic in the framework of M-R studies (Fattorini et al. 2007a), Fattorini et al. (2007b) demonstrated that both may be relaxed and BOWE can constitute a reliable and robust method for estimating population size, provided that marks are distributed quite evenly among groups. The tendency for a solitary behaviour in male Alpine chamois during summer (Krämer 1969), supported by the consistently low mean group size observed during distance sampling (1.23 ± SD 0.043), therefore suggests that M-R using BOWE may possibly represent a fairly robust method to estimate the numerical size of our population, as there should be no or few groups and hence there should be no uneven distribution of marks among them.

Indeed, the M-R based on 5 subsequent sessions of resighting from fixed points and paths, with the aid of optical instruments, possibly gave accurate estimates of the population size, as it was the sole method whose confidence intervals included the minimum number of males alive in the population. Furthermore, despite the profound differences in the sampling protocols, similar results (though with wider confidence intervals) were obtained applying BOWE to the distance sampling data, therefore supporting the robustness of the M-R estimates. The difference between the two estimates were indeed attributable to sampling variability.

Such M-R based estimates yielded lower levels of precision (SE estimates of 18% and 24%, respectively), compared to DS (SE estimate of 14%). Yet, a precision of 18 % ensures a quite reliable monitoring of the population changes: suppose a population of 90 individuals at time 1 and a mark-resight experiment repeated exactly at times 1 and 2 (i.e. with the same marked animals, observers, number of sessions, period, hours, paths, and optical instruments) so that the same precision of 18% and a strong correlation of 0.9 can be realistically presumed for the estimates achieved at the two occasions. A directional t-test performed at significance level $\alpha=0.10$ on the difference between estimates, is able to detect an actual population decrease of 20% (corresponding to 18 individuals) with a power of about 0.9 and an actual population increase of 20% with a power of about 0.8. Higher levels of precision (and power) require an increase of marked individuals and of resighting occasions (Diefenbach 2009).

Distance sampling

Previous studies have compared size estimates of chamois populations obtained with DS and other methods. García-González et al. (1992) evaluated the density of Pyrenean chamois *Rupicapra p. pyrenaica* populations using M-R and line DS and they obtained lower but more precise estimated with DS, as in our results. Herrero et al. (2011) used BC and point DS on the same species: DS data were collected from 15-23 transects for 119-199 km, they obtained SE estimates ranging between 26% and 15%, and the confidence interval of DS included the number of animals observed with BC. Similarly, López-Martin et al. (2013) found an overabundance in BC when compared with line DS, but the numbers obtained with BC were always within the 95% confidence interval of DS.

Our sampling effort was sufficient to obtain quite precise estimates (SE of about 14%), confirming what suggested by Buckland et al. (2001) who recommend at least 60-80 samples. Yet, the abundance estimate obtained using DS was biased low, as the upper limit of the confidence interval was lower than the minimum number of males alive in the population. This bias finds confirmation in the large positive difference between M-R and DS estimates, which is not attributable to sampling variability. While the accuracy of distance measures was accomplished thank to the use of precise instruments, with our data and results we are not able to draw certain conclusions about the respect of other assumptions. As stated above, the landscape complexity and the rough nature of the Alpine terrain did not allow a random distribution of the transects. As a result, we probably obtained that animals were not equally approachable from the transects and this may have caused a bias in chamois abundance estimate (Marques et al. 2010). For such circumstances, new approaches are recently being developed. Marques et al. (2013) proposed a method that takes into account the density gradient with respect to linear features, such as roads, trails and fences, when implementing analyses with distance sampling. In particular they used a sample of GPS-collared animals to build a spatial density model that was used to correct the density estimator. Although our animals were indeed GPS-collared, only a subset of the 14 GPS devices were still working by the time of the surveys, so we were able to localize some of them by GPS and some of them by VHF. Because the precision and frequency of fixes collected during surveys were different for the two methods, this lack of homogeneity did not allow us to perform robust analyses on trail avoidance by male chamois. In general, however, it is always recommendable, if possible, to investigate animal distribution around linear features that are not randomly located, before the experimental design of the survey (Erxleben et al. 2011).

A valid alternative to line transect sampling could be the use of point transect sampling, because in a mountainous landscapes it would be less hard reaching random points than travelling random transects (e.g. Franceschi et al. 2014). Yet, in many mountainous areas it may be sometimes difficult to reach all randomly-distributed points, for example due to the presence of cliffs: in that case the assumption that animals are equally approached from the points in the whole surveyed area would likely be violated again, thus leading to a biased estimation of density (Marques et al. 2010). Recently, new methods and tools have been implemented to account for animal gradient along parallel contour or when density varies with an environmental variable, in point transect density estimation (Cox et al. 2013). These new methods were implemented specifically for sea animals but it would be worth studying their applicability even to mountain-dwelling species.

Conclusions

Estimating the population size of mountain-dwelling ungulates remains a challenging issue. Due to the rugged nature of the mountainous terrain, block counts are likely to underestimate population abundance, and line distance sampling appears poorly suited to estimate the size of alpine ungulate populations. DS estimate was negatively biased even if it achieved a good level of precision (SE estimate of 14%). In such an environment, point distance sampling may be a suitable alternative, as it should offer better visibility conditions and better estimates of the sampled area. Even if BOWE provided less precise estimates than DS (SE estimate of 18%), it is expected to be accurate because marks were fairly evenly distributed among animals. In turn, this suggests that mark-resight methods may represent fairly robust alternatives to estimate chamois population size. Accuracy is of primary importance when estimating abundance: if an estimator is greatly biased, it is of poor consolation that its variance is low. Given the relatively small size of the target population, increasing the number of marked animals and/or resighting occasions may possibly help to obtain more precise M-R estimates.

Acknowledgments

We are grateful to the wardens of the PNGP for their effort in darting chamois, and for their help in collecting data with the block counts. We thank B. Bassano (GPNP) for his help in capturing chamois and for promoting the development of this study and C. Lorenzetti for her help during mark-resight surveys. We thank S. Lovari (University of Siena) for his support throughout the study on chamois in the GPNP, and J. Herrero (University of Zaragoza) for helpful comments on earlier drafts of this manuscript. Part of this work was funded within the framework of GREAT (Large herbivores in Changing Alpine Ecosystems), a project co-funded by the ERDF (European Regional Development Fund) through the cross-border cooperation programme Interreg IVA Italy-Switzerland 2007-2013.

References

Arnason, A. N., Schwarz, C. J., and Gerrard, J. M. (1991). Estimating closed population size and number of marked animals from sighting data. Journal of Wildlife Management, *55*, 716-730.

Aublet, J. F., Festa-Bianchet, M., Bergero, D., and Bassano, B. (2009). Temperature constraints on foraging behaviour of male Alpine ibex (*Capra ibex*) in summer. Oecologia, *159*, 237-247.

Baccaro, F. B., and Ferraz, G. (2012). Estimating density of ant nests using distance sampling. Insectes Sociaux, *60*, 103-110.

Bächler, E., and Liechti, F. (2007). On the importance of g(0) for estimating bird population densities with standard distance-sampling: implications from a telemetry study and a literature review. Ibis, *149*, 693-700.

Barlow, J. (2006). Cetacean abundance in Hawaiian water estimated from a summer/fall survey in 2002. Marine Mammal Science, *22*, 446-464.

Bartmann, R. M., White, G. C., Carpenter, L. H., and Garrott, R. A. (1987). Aerial mark-recapture estimates of confined mule deer in pinyon-juniper woodland. Journal of Wildlife Management, *51*, 41-46.

Bowden, D. C., and Kufeld, R. C. (1995). Generalized mark-sight population size estimation applied to Colorado moose. Journal of Wildlife Management, *59*, 840-851.

Buckland, S. T., Goudie, I. B. J., and Brochers, D. L. (2000). Wildlife population assessment: past developments and future directions. Biometrics, *56*, 1-12.

Buckland, S. T., Anderson, D. R., Burnham, K. P., Laake, J. L., Borchers, D. L., and Thomas, L. (2001). Introduction to Distance Sampling. Oxford University Press, Oxford

Buckland, S. T., Anderson, D. R., Burnham, K. P., Laake, J. L., Borchers, D. L., and Thomas, L. (2004). Advanced Distance Sampling. Oxford University Press, Oxford

Buckland, S. T., Plumptre, A. J., Thomas, L., and Rexstad, E. A. (2010). Design and analysis of line transect surveys for primates. International Journal of Primatology, *31*, 833-847.

Chirichella, R., Ciuti, S., and Apollonio, M. (2013). Effects of livestock and non-native mouflon on use of high-elevation pastures by Alpine chamois. Mammalian Biology, *78*, 344-350.

Corlatti, L., Lorenzini, R., and Lovari, S. (2011). The conservation of the chamois *Rupicapra* spp. Mammal Review, *41*, 163-174.

Corlatti, L., Caroli, M., Pietrocini, V., and Lovari, S. (2013a). Rutting behaviour of territorial and nonterritorial male chamois: is there a home advantage? Behavioural Processes, *92*, 118-124.

Corlatti, L., Bassano, B., Valencak, T. G., and Lovari, S. (2013b). Foraging strategies associated to alternative reproductive tactics in a large mammal. Journal of Zoology, *291*, 111-118.

Corlatti, L., and Bassano, B. (2014). Contrasting alternative hypotheses to explain rut-induced hypophagia in territorial male chamois. Ethology, *120*, 32-41.

Cox, M. J., Borchers, D. L., and Kelly, N. (2013). nunpoint: an R package for density estimation from point transects in the presence of nonuniform animal density. Methods in Ecology and Evolution, *4*, 589-594.

Dematteis, A., Menzano, A., Canavese, G., Meneguz, P. G., and Rossi, L. (2009). Anaesthesia of free-ranging Northern chamois (*Rupicapra rupicapra*) with xylazine/ketamine and reversal with atipamezole. European Journal of Wildlife Research, *55*, 567-573.

Diefenbach, D. R. (2009). Estimating avian population size using Bowden's estimator. The Auk, *126*, 211-217.

Erxleben, D., Butler, M., Ballard, W., Wallace, M., Peterson, M., Silvy, N., Kuvlesky, W., Hewitt, D., DeMaso, S., Hardin, J., and Dominguez-Brazil, M. (2011). Wild turkey (*Meleagris gallopavo*) association to roads: implications for distance sampling. European Journal of Wildlife Research, *57*, 57-65.

Fattorini, L., Marcheselli, M., Monaco, A., and Pisani, C. (2007a). A critical look at some widely used estimators in mark-resighting experiments. Journal of Animal Ecology, *76*, 957-965.

Fattorini, L., Marcheselli, M., Monaco, A., and Pisani, C. (2007b). Estimating population size by means of mark–resighting counts: theoretical considerations and empirical results. Working Paper no.70. Dipartimento di Metodi Quantitativi, Università di Siena, Siena, Italy.

Franceschi S, Nelli L, Pisani C, Franzoi A, Fattorini L, Meriggi A (2014) A Monte Carlo appraisal of plot and distance sampling for surveys of black grouse and rock ptarmigan populations in Alpine protected areas. J Wildl Manag (in press)

Furlow RC, Haderlie M, Van den Berge R (1981) Estimating a bighorn sheep population by mark-recapture. Desert Bighorn Council Transactions 25:31-33

Gaillard J-M, Loison A, Toïgo C (2003) Variation in life history traits and realistic population models for wildlife management: the case of ungulates. In: Festa-Bianchet M, Apollonio M (eds) Animal behavior and wildlife conservation. Island Press, Washington, pp 115-132

García-González R, Hidalgo R, Ameztoy JM, Herrero J (1992) Census, population structure and habitat use of a chamois population in Ordesa N.P. living in sympatry with Pyrenean wild goat. In: Spitz F, Janeau G, Gonzalez G, Alaugnier S (eds) Ongulés/Ungulates 91. SFEPM-IRGM, Toulouse, pp 321-325

Garel M, Bonenfant C, Hamann J-L, Klein F, Gaillard J-M (2010) Are abundance indices derived from spotlight counts reliable to monitor red deer *Cervus elaphus* populations? Wildl Biol 16:77-84

Greenwood JJD, Robinson RA (2007) General census methods. In: Sutherland WJ (ed) Ecological census techniques: a handbook. Cambridge University Press, Cambridge, pp 87-185

Hein EW, Andelt WF (1995) Estimating coyote density from mark-resight surveys. J Wildl Manag 59:164-169

Herrero J, García-Serrano A, Prada C, Fernández-Arberas O (2011) Using block counts and distance sampling to estimate populations of chamois. Pireneos 166:123-133

Houssin H, Loison A, Gaillard J-M, Jullien J-M (1994) Validité d'une méthode d'estimation des effectifs de chamois dans un massif des Préalpes du nord. Gibier Faune Sauvage 11:287-298

Krämer A (1969) Soziale organisation und sozialverhalten einer gemspopulation *Rupicapra rupicapra* der Alpen. Z Tierpsychol 26:889-964

Jacobson AR, Provenzale A, von Hardenberg A, Bassano B, Festa-Bianchet M (2004) Climate forcing and density dependence in a mountain ungulate population. Ecology 85:1598-1610

La Morgia V., Bassano B (2009) Feeding habits, forage selection and diet overlap in Alpine chamois (*Rupicapra rupicapra* L.) and domestic sheep. Ecol Res 24:1043-1050

Largo E, Gaillard J-M, Festa-Bianchet M, Toïgo C, Bassano B, Cortot H, Farny G, Lequette B, Gauthier D, Martinot J-P (2008) Can ground counts reliably monitor ibex *Capra ibex* populations? Wildl Biol 14:489-499

Lettink M, Armstrong DP (2003) An introduction to using mark-recapture analysis for monitoring threatened species. Department of Conservation Technical Series 28A:5-32

Lloyd-Smith JO, Cross PC, Briggs CJ, Daugherty M, Getz WM, Latto J, Sanchez MS, Smith AB, Swei A (2005) Should we expect population thresholds for wildlife disease? Trends Ecol Evol 20:511-519

Loison A, Appolinaire J, Jullien J-M, Dubray D (2006) How reliable are total counts to detect trends in population size of chamois *Rupicapra rupicapra* and *R. pyrenaica*? Wildl Biol 12:77-88

López-Martín JM, Xifra J, Serrano E (2013) Estimating the population density of Pyrenean chamois using distance sampling methods. II Rupicapra Symposium, 24 – 25 October 2013. Bellver de Cerdanya, Catalonia, Spain

Lovari S, Sacconi F, Trivellini G (2006) Do alternative strategies of space use occur in male Alpine chamois? Ethol Ecol Evol 18:221-231

Marques TA, Buckland ST, Borchers DL, Tosh D, McDonald RA (2010) Point transect sampling along linear features. Biometrics 66:1247-1255

Marques TA, Buckland ST, Bispo R, Howland B (2013) Accounting for animal density gradient using independent information in distance sampling surveys. Stat Meth Appl 22:67-80

McClintock BT, White GC, Burnham KP (2006) A robust design Mark-Resight abundance estimator allowing heterogeneity in resighting probabilities. J Agr Biol Environ Stat 11:231–248

McClintock BT, White GC, Burnham KP, Pryde MA (2009) A generalized mixed effects model of abundance for mark-resight data when sampling is without replacement. In: Thomson DL, Cooch EG, Conroy MJ (eds) Modeling demographic processes in marked populations. Springer, New York, pp 271-289

McClintock BT, White GC, Antolin MF, Tripp DV (2009b) Estimating abundance using Mark–Resight when sampling is with replacement or the number of marked individuals is unknown. Biometrics 65:237-246

Miller SD, Becker EF, Ballard WH (1987) Black and brown bear density estimates using modified capture-recapture techniques in Alaska. International Conference on Bear Reasearch and Management 7:23-35

Minta S, Mangel M (1989) A simple population estimate based on simulation for capture-recapture and capture-resight data. Ecology 70:1738-1751

Morellet N, Gaillard J-M, Hewison AJM, Ballon P, Boscardin Y, Duncan P, Klein F, Maillard D (2007) Indicators of ecological change: new tools for managing populations of large herbivores. J Appl Ecol 44:634-643

Neal AK, White GC, Gill RB, Reed DF, Olterman JH (1993) Evaluation of mark-resight model assumptions for estimating mountain sheep numbers. J Wildl Manag 57:436-450

Newey S, Bell M, Enthoven S, Thirgood S (2003) Can distance sampling and dung plots be used to assess the density of mountain hares *Lepus timidus*? Wildl Biol 9:185-192

Nussberger B, Ingold P (2006) Effects of radio-collars on behaviour of Alpine chamois *Rupicapra rupicapra rupicapra*. Wildl Biol 12:339-343

Otis DL, Burnham KP, White CW, Anderson DR (1978) Statistical inference from capture data on closed animal populations. Wildl Monogr 62:3-135

Pérez JM, Serrano E, Alpizar-Jara R, Granados JE, Soriguer RC (2002) The potential of distance sampling methods to estimate abundance of mountain ungulates: review of usefulness and limitations. Pirineos 157:15-23

Pollock KH, Nichols JD, Simons TR, Farnsworth GL, Bailey LL, Sauer JR (2002) Large scale wildlife monitoring studies: statistical methods for design and analysis. Environmetrics 13:105-119

Rice WR, Harder JD (1977) Application of multiple aerial sampling to a mark-recapture census of white-tailed deer. J Wildl Manag 41:197-206

Ries EH, Hiby LR, Reijnders PJH (1998) Maximum likelihood population size estimation of harbour seals in the Dutch Wadden Sea based on capture-recapture experiment. J Appl Ecol 35:332-339

Rughetti M, Toïgo C, von Hardenberg A, Rocchia E, Festa-Bianchet M (2011) Effects of an exceptionally snowy winter on chamois survival. Acta Theriol 56:329-333

Schröder W, von Elsner-Schack IV (1985) Correct age determination in chamois. In: Lovari S (ed) The biology and management of mountain ungulates. Croom Helm, London, pp. 67-70

Schwarz CJ, Seber GAF (1999) Estimating animal abundance: review III. Stat Sci 14:427-456

Seber GAF (1982) The estimation of animal abundance and related parameters, 2nd edition. Griffin, London

Sinclair ARE, Fryxell JM, Caughley G (2006) Wildlife ecology, conservation and management, 2nd edition. Blackwell Publishing, Oxford

Stephens PA, Sutherland WJ, Freckleton RP (1999) What is the Allee effect? Oikos 87:185-190

Thomas L, Buckland ST, Rexstad EA, Laake JL, Strindberg S, Hedley SL, Bishop JRB, Marques TA, Burnham KP (2010) Distance software: design and analysis of distance sampling surveys for estimating population size. J Appl Ecol 47:5-14

Willisch CS, Bieri K, Struch M, Franceschina R, Schnidrig-Petrig R, Ingold P (2013) Climate effects on demographic parameters in an unhunted population of Alpine chamois (*Rupicapra rupicapra*). J Mammal 94:173-182

White GC (1993) Evaluation of radio tagging marking and sighting estimators of population size using Monte Carlo simulations. In: Lebreton JD, North PM (eds) Marked Individuals in the Study of Bird Populations. Birkhauser Verlag, Basel, pp 91-103

White GC (1996) NOREMARK: Population estimation from mark-resighting surveys. Wildl Soc Bull 24:50-52

White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked individuals. Bird Study 46:120-139

Wingard GJ, Harris RB, Amgalanbaatar S, Reading RP (2011) Estimating abundance of mountain ungulates incorporating imperfect detection: argali *Ovis ammon* in the Gobi Desert, Mongolia. Wildl Biol 17:93-101

Yoccoz NG, Nichols JD, Boulinier T (2001) Monitoring of biological diversity in space and time. Trends Ecol Evol 16:446-453