Research Article

A comparison of four methods to estimate population size of Alpine marmot (*Marmota marmota*)

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Abstract

Obtaining reliable information on animal abundance in mountainous landscapes is challenging. Highly heterogeneous habitats tend to reduce detection probabilities, and the three-dimensional, rugged nature of the terrain poses severe limits to the fulfilment of a number of assumptions underlying several statistical methods. In this study, we compared the performance of 4 methods to estimate population size of Alpine marmot (*Marmota marmota*), a highly social semifossorial rodent widely distributed on the European Alps. Between May and August 2015, in a study area within the Stelvio National Park (Italy), we conducted 8 sessions of capture-mark-recapture, 6 sessions of mark-resight from vantage points, 8 sessions of line distance sampling along 4 transects, and 2 sessions using double-observer methods from vantage points. The minimum number of animals alive, obtained during the mark-resight surveys, was n=54 individuals. Capture-mark-recapture models estimated a population size of n=56 individuals (95% CI=45–87); similar, but more precise estimates were obtained with the mark-resight approach (Bowden's estimator: n=62, 95% CI=54–71; Poisson log-normal estimator: n=62, 95% CI=55–69). Line transect distance sampling and double-observer methods were severely biased low (line transect distance sampling: n=24 individuals, 95% CI=19–31; independent double-observer: n=24, 95% CI=22–35; dependent double-observer: n=15, 95% CI=15–20). Our results suggest that the probabilistic approach based on marked individuals yielded fairly robust estimates of population size. The underestimates obtained using distance sampling and double-observer methods were likely due to the violation of some underlying assumptions. While the topography of the mountainous landscape makes it difficult to randomize the distribution of line transects, the burrowing behaviour of the species is likely to violate the assumption of perfect detection on the transect; in addition, the semifossorial habits of marmots lower their detection probabilities, thus hampering the performance of these methods.

Introduction

Reliable information on population size is often of timely importance for the conservation and management of wildlife populations (Sinclair et al., 2006). While over-time changes in abundance may be reliably investigated with appropriate indexes (Morellet et al., 2007), good abundance estimates often require the use of statistical methods that account for non-perfect detection. To this end, several estimators have been developed, either based on the availability of marked individuals (e.g. capture-mark-recapture — CMR: Otis et al., 1978; mark-resight — MR: Schwarz and Seber, 1999) or unmarked individuals (e.g. distance sampling — DS: Buckland et al., 2001; double-observer methods — DO: Nichols et al., 2000). The goodness of the estimates crucially relies on the possibility to meet all the assumptions underlying each estimator, and the choice of the most appropriate methodology largely depends upon the behavioural characteristics of the target species and on the operative constraints imposed by field conditions.

Mountainous landscapes pose several challenges for the estimation of population abundance (Singh and Milner-Gulland, 2011). Heterogeneous habitats tend to reduce detectability rates, and the three-dimensional, rough nature of the mountainous terrains often limits the possibility to successfully meet critical assumptions of several statistical methods. Random distribution of transects or observation points,

for example, can be severely constrained by the presence of virtually inaccessible areas such as cliffs or rocks (Corlatti et al., 2015). The behaviour of animals may also play a key role in the choice of the most appropriate method: semifossorial habits, for example, hinder the detection probability, and social aspects such as the tendency to form groups or defend territories may cause serious violations of assumptions such as independence of detections in space and in time (Fattorini et al., 2007). A proper evaluation of the assumptions underlying each methodology is therefore required when accounting for non-perfect detection.

If a subset of the unknown population is marked, a capture-mark-recapture approach can be used to estimate detection probabilities and population size (Williams et al., 2002). The CMR methodology has been widely applied to several taxa such as raptors (Gould and Fuller, 1995), carnivores (Sharma and Jhala, 2011; Gerber et al., 2014) and rodents (Jareño et al., 2014), including black-tailed prairie dogs (*Cynomys ludovicianus*) (Severson and Plumb, 1998). In its simplest form, the CMR approach implies that animals are captured on a first occasion and released back into the population. The population is then re-sampled in a subsequent occasion and the proportion of marked individuals is used to estimate population size. Multiple capture occasions can be used to obtain more reliable estimates (Chao and Huggins, 2005). Closed CMR methods crucially assume that the target population remains numerically stable during surveys and marks are not lost. Classic closed CMR estimators also require constant and equal capture

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probability among animals (Chao and Huggins, 2005). As this latter assumption is fairly unrealistic, Otis et al. (1978) proposed a suite of models that may account for time-variation over trapping occasions, behavioural response (trap-shyness, trap-happiness) after the first capture, and individual heterogeneity among animals. All these models can be implemented in program MARK (White and Burnham, 1999).

Likewise, mark-resight methods have been used to estimate population abundance in several *taxa*, including the semifossorial black-tailed prairie dog (Magle et al., 2007), and proved efficient in estimating population size of mountain-dwelling populations of ungulates (Corlatti et al., 2015). MR slightly differs from CMR in that recapture events are replaced by resightings, and thus offers several advantages because fieldwork is normally less expensive and less invasive (McClintock and White, 2012). All MR models share the basic assumption that marks must not be lost during surveys; several other assumptions (e.g. demographic closure, sampling with or without replacement, homogeneous probability of resighting, independence among resightings) are specific to each estimator. Earlier MR estimators include 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) implemented in NOREMARK (White, 1996). More recent, likelihood-based models include the Logit-Normal Estimator (LNE), the Immigration-Emigration Logit Normal Estimator (IELNE) and the Poisson log-Normal Estimator (PNE) (McClintock and White, 2012) implemented in MARK (White and Burnham, 1999).

Wildlife managers, however, are often required to run long-term monitoring programs on large scale, for which the aforementioned methods are unsuitable, owing to the costs of captures. When marked individuals are not available, the distance sampling method (DS) can represent a valid alternative to estimate population density or abundance. In its general formulation, DS can be considered as an extension of the plot sampling method in which the probability of detection is estimated according to the distribution of the distances of objects from the observer (Buckland et al., 2001). The most widely used form of DS is the line transect sampling, in which a survey region is sampled recording any animal observation along lines, placed randomly or systematically spaced, within a truncation distance w. The method is based on the definition of a detectability function $g(x)$ that describes the probability to detect an animal as a function of its perpendicular distance x from the transect, assuming that all the animals on the lines are detected and that the detectability decreases with increasing distance. The function $g(x)$ allows to estimate the probability P of detecting an animal within the distance w, and the animal density can be estimated as $D = n/(P \times 2wL)$, where n is the number of detected animals and L the total length of the transects. The animals can be single individuals or clusters of individuals. In the latter case, the method gives a density of clusters, and the individual density must be estimated, generally as a mean of individuals for cluster or by using a regression method in which cluster size is regressed on estimated probability of detection (Thomas et al., 2010). The function $g(x)$ is not known a priori, but it is usually modelled by combining key functions with series expansions, if needed, while statistical methods allow to choose the best model that fits the observed data. Further options of the method consider the effect of covariates, such as habitat parameters or weather conditions, that may affect the detection function.

The double-observer method can also be applied when marks are not available. This approach found application in *taxa* as diverse as birds (Nichols et al., 2000), bats (Duchamp et al., 2006) and ungulates (Jenkins and Manly, 2008). The DO methods can be distinguished in: independent double-observer (IDO) and dependent double-observer (DDO). In IDO a Lincoln-Petersen-like capture-recapture framework can be adopted even in the absence of actual captures, thanks to the detection of animals by one or both observers, but this method can be extended to multiple observers, allowing individual heterogeneity to be modelled (Williams et al., 2002). A capture history can be built for each observed individual and data can be analysed in a CMR-like fashion (Williams et al., 2002). IDO assumes that the population must be closed during surveys, counts are independent, animals can be identified, and each sighting represents a simple random sample of the entire population (Magnusson et al., 1978; Suryawanshi et al., 2012). In the DDO approach, two observers are designated as primary and secondary and counts are not independent. The two observers can switch roles multiple times during the survey, and by the end of the count the data will consist of the number of animals detected and missed by the primary observer (Williams et al., 2002). These data will serve as a basis for the estimation of observer-specific detection probabilities and, in turn, population size. The DDO approach is most likely to succeed when detection probability is high and the two observers have similar abilities of detecting animals (Nichols et al., 2000). Both methods are implemented in program DOBSERV (Nichols et al., 2000).

The Alpine marmot (*Marmota marmota*) is a highly social, semifossorial rodent inhabiting the high-elevation mountainous areas of Central and Southern Europe. Social groups are composed by a dominant couple with offspring and helpers (Perrin et al., 1993), and each family group defends a territory that includes its burrow system. Despite its wide distribution and abundance on the Alps, little information is available about marmot density and about the performance of different estimate methodologies (Pelliccioli and Ferrari, 2013): absolute population density and spatial distribution of Alpine marmot have been mainly estimated using live trapping (capture-recapture models; Allainé et al., 1994; Farand et al., 2002), which also allows individual identification from distance (Ranghetti, 2009). Alternative methods for density estimation include the use of point-transects distance sampling (Pelliccioli and Ferrari, 2013). Minimum density values can be obtained by multiplying the mean size of marmot nucleus by the number of nuclei present in the study area (e.g. Borgo et al., 2008; see Barrio et al., 2013 for a comprehensive review of the available studies). In this study we aim to compare the performance of the four methods described above (CMR, MR, DS and DO) to estimate the absolute adult (i.e. ⩾1 year of age) population size of the Alpine marmot in a study area within the Stelvio National Park, Central Italian Alps.

Materials and methods

Study area and population

The study site is located in Malga Levi (46°22′ N, 10°43′ E) in Val de la Mare, a side valley of the Peio valley, within the Stelvio National Park (Province of Trento, central Italian Alps). The climate is harsh and highly variable, as typical of high-elevation mountains, with years of large snowfalls. Between 1990 and 2007 the average daytime temperature ranged from 8.3 °C in July to −7.3 °C in January and snow depth in winter (December-March) was 62 ± 27 cm (mean±SE) (data obtained from Careser Dam meteorological station, Peio valley, 2595 m a.s.l.: Bassan, 2006). The study area ranges between 2120 and 2520 m a.s.l. and extends over 68 ha. According to live trapping activities, 25 and 42 marmots were captured and marked in 2014 and 2015, respectively. Mark-resight and distance sampling sessions (see below) have been used, together with opportunistic daily sighting sessions of marked animals in the second part of the summer, for estimating the borders and the size of family group territories, as well as the minimum number of marked individuals belonging to each family. The study population consists of 6 family-group territories (mean elevation: 2296 ± 76 m; mean HR size: 2.04 ± 0.64 ha) with different exposures (north-facing slopes and valleys, but mainly south-facing slopes). According to the observations of marked animals, the minimum average family group size in 2015 was 8.5 ± 2.3 individuals. Territories are situated in a glacial cirque dominated by alpine grasslands of Alpine sedge (*Carex curvula*) and Haller's fescue (*Festuca halleri*), siliceous screes, Vaccinium-Rhododendron shrubs and steep rocky slopes; the lower part of the study site is bordered by forests of larch (*Larix decidua*) and spruce (*Picea abies*).

Capture-mark-recapture

At the beginning of the study, between May 7 and May 14, 2015, we conducted 8 secondary sessions of live-trapping using two-door Toma-
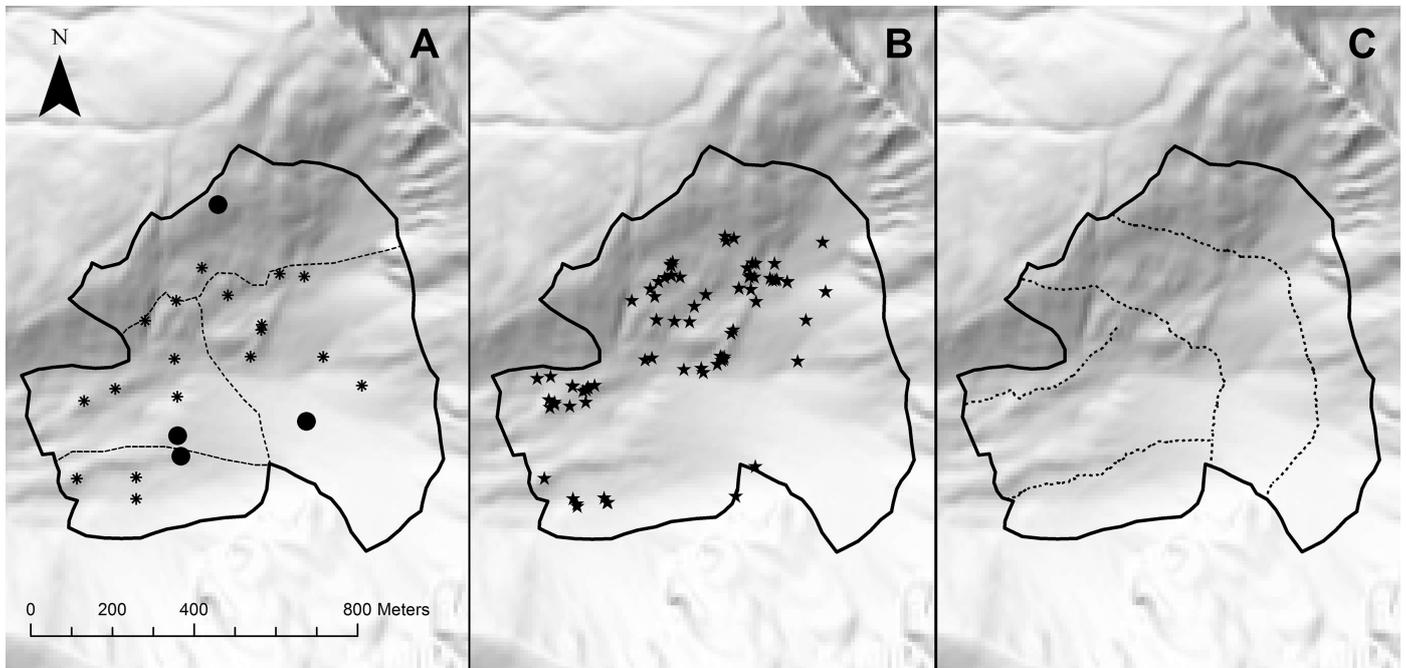
Figure 1 – a) Distribution of the box-traps used during the capture-mark-recapture sessions (asterisk), sectors and vantage points used during the sessions of mark-resight and double-observer (point). b) Distribution of marked individuals over the study site (stars); data were obtained pooling all observations of marked animals spotted during the 6 mark-resight sessions. c) Line transects (dashed lines) used for distance sampling.

hawk's traps (n=18) distributed over the entire study site (Fig. 1a), and baited with dandelion flowers (*Taraxacum officinalis*), apples, carrots and celery. Traps were kept open between 6 a.m and 9 p.m. every day, for a total of 8 days; catching effort was constant throughout the 8 secondary sessions. After each capture event, traps were re-baited. Over the entire primary session we captured n=39 marmots; each individual was assigned to a given sex- and age-class (young, adult male, adult female; kids were not captured because trapping occurred before the birth period): sex was determined through inspection of the ano-genital morphology (Zelenka, 1965), while biometric measurements (length, body mass) were used to distinguish age classes. Prior to release, each individual was marked permanently by injection of a Tracer Bayer transponder pit and – for visual identification – with different combinations of coloured ear-tags. During captures, animals were not sedated and the entire manipulation process always took less than 20 min. These methods are in accordance with the Italian law, as captures were made after receiving authorization from the Autonomous Province of Trento in accordance with ISPRA (the Institute for Environmental Protection and Research) and with the assistance of a veterinarian. At the end of the 8 secondary sessions, for each individual we built a capture history (1/0) depending on the occurrence of capture-recapture events.

Because captures were conducted over a short time frame, we adopted a closed-population assumption (Otis et al., 1978). We thus fitted a suite of 8 closed-population models to account for variation in encounter probability (White, 2008), using the terminology of Otis et al. (1978): M_0 (constant capture probabilities), M_t (capture probabilities vary with time), M_b (capture probabilities vary by behavioural response to capture), M_h (capture probabilities vary by individual animal), M_{tb} (capture probabilities vary by time and behavioural response to capture), M_{th} (capture probabilities vary by time and individual animal), M_{bh} (capture probabilities vary by individual animal and by behavioural response to capture), M_{tbh} (capture probabilities vary by behavioural response to capture, time and individual animal). Data were analysed using the package RMark (Laake, 2013) with R 3.1.3 (R Development Core Team, 2015) in R Studio 0.99.446 (RStudio, 2015), using the full likelihood approach and a 2-mixtures modelling to account for individual heterogeneity (White, 2008). We compared the models by means of the AIC-based model selection approach; model averaging accounted for selection uncertainty, using a cut-off value of

ΔAICc (Akaike's Information Criterion corrected for small samples) <7 to select competing models (Burnham and Anderson, 2002).

Mark-resight

Over 4 different days, between May 25 and June 4, 2015, we conducted 6 secondary sessions of resighting (4 in the morning hours between 10 a.m. and 12 a.m., 2 in the afternoon between 3 p.m. and 5 p.m.). In each session, the entire study area was divided into 4 sectors consecutively surveyed from 4 different vantage points (Fig. 1a) by two observers (MB, 2 sectors; FZ, 2 sectors) using binoculars 8.5×42 to spot animals, and spotting scopes 20–60×65 to confirm the presence of ear-tags and identify individuals. During each survey we recorded the number of unmarked individuals, the number of unidentified marked individuals, the number and identity of individually recognized marmots, the size of groups observed and the number of marked individuals within each group. Observations of kids were discarded to avoid bias in the estimate. During surveys, we also recorded the position of marked individuals using the animal locator method (Pasquaretta et al., 2012), to confirm that marks were fairly evenly distributed over the study area (Fig. 1b).

As we did for the CMR, all the resighting sessions during MR were conducted over a short time frame, thus we adopted a closed-population assumption (Otis et al., 1978). Because the MR surveys started soon after the end of the CMR, the number of marked individuals available for resighting was assumed to be n=39. To estimate population size we used BOWE implemented in NOREMARK, which proved to be a robust MR estimator provided marks are evenly distributed among groups (Fattorini et al., 2007). BOWE relaxes several assumptions of MR, as it does not require models, it allows for heterogeneity in resighting probability and it does not assume independence among sighting trials (Bowden and Kufeld, 1995). Furthermore, BOWE allows for the inclusion of unidentified marked individuals in the estimate, provided the identification process for marked animals satisfies additional conditions. Specifically, all identification trials (i.e. the attempts to determine the individual identity of marked animals) should be treated as independent trials with identical probabilities of success (for details see Bowden and Kufeld, 1995 p. 844). For the sake of comparison, we also attempted an estimate of population size using the PNE implemented in MARK (McClintock et al., 2009). PNE also allows for heterogeneity in resighting probability.

Table 1 – Summary information about the different methods (capture-mark-recapture - CMR, mark-resight - MR, line transect distance sampling - DS, dependent double-observer - DDO and independent double-observer - IDO) used to estimate the population size of Alpine marmot in the study site within the Stelvio National Park in spring-summer of 2015.

	CMR	MR	DS	DDO	IDO
Number of operators	2	2	2	2	2
Dates	7/5–14/5	25/5–4/6	3/8–13/8	30/6	4/7
Time	6–21	10–12 / 15–17	9–12 / 17–19	9–11	16–18
Number of days	8	4	6	1	1
Number of occasions	8	6	8	1	1
Sampling protocol	18 box-traps	4 sectors from points	4 line transects	4 sectors from points	4 sectors from points
Optical instruments	no	yes	yes	yes	yes
Marmots seen/captured	n=39	n=173	n=269	n=15	n=21

Unlike BOWE, however, it requires independently and identically distributed resighting probabilities for each animal, an assumption that is often difficult to meet in the field (Fattorini et al., 2007). PNE also allows for the inclusion of unidentified marked individuals in the estimate (McClintock et al., 2009 p. 236). Furthermore, in PNE both the resighting probability α and the individual heterogeneity σ can be modelled as a function of individual covariates and σ can be also set to zero (no individual heterogeneity). We thus used the package RMark (Laake, 2013) with R 3.1.3 (R Development Core Team, 2015) in R Studio 0.99.446 (RStudio, 2015) to fit 6 models with different parameterizations of α and σ: $\alpha(.)\sigma(0)$ (constant resighting probabilities without individual heterogeneity); $\alpha(.)\sigma(.)$ (constant resighting probabilities with individual heterogeneity); $\alpha(.)\sigma(cov)$ (constant resighting probabilities with heterogeneity as a function of individual covariates); $\alpha(cov)\sigma(0)$ (resighting probabilities as a function of individual covariates without individual heterogeneity); $\alpha(cov)\sigma(.)$ (resighting probabilities as a function of individual covariates with individual heterogeneity); $\alpha(cov)\sigma(cov)$ (resighting probabilities and heterogeneity as a function of individual covariates). We used the sex- and age-classification of marked animals (young, adult male, adult female) as individual covariate. The models were then compared using an AIC-based model selection approach; model averaging was used to account for model selection uncertainty and a cut-off value of $\Delta AICc<7$ was adopted to select competing models (Burnham and Anderson, 2002).

Line transect distance sampling

We placed 4 linear transects with an average length of 760 m, according to an opportunistic design, in order to cover the whole study area. Due to the terrain ruggedness, two out of 4 transects followed isopleths (Fig. 1c). From 3 to 13 August 2015, two operators alternatively walked the transects early in the morning or late in the afternoon. Each transect was repeated 8 times, for an overall effort of 24.3 km. Perpendicular distances from the transect were collected for each observation of single individual or groups of individuals with a laser rangefinder. Animals that were seen out of the line of the transect, i.e. animals for which the perpendicular projection was out of the transect, were excluded from the analysis. As for the MR methods, observations of kids were discarded to avoid bias in the estimate.

To analyse the data, we first used the conventional distance sampling engine of the Distance software (CDS). We performed estimation starting from uniform, half-normal and hazard-rate key functions with cosine, simple and Hermite polynomial series adjustment. For the selection of adjustment terms we used the default settings of the software, i.e. a sequential automated selection based on the AICc values. We used the exact distances and discarded the largest 5% observations. To validate the models we used the Kolmogorov-Smirnov goodness-of-fit statistics (GOF K-S) and we ranked them according to their AICc value. We then used the multiple covariate engine (MCDS) (Marques et al., 2007) including the effect of operator, disturbance, weather and habitat composition to investigate the role of covariates on the estimation of the detection function. The encounter rate variance was estimated empirically; for the cluster size estimation we used the mean of observed clusters.

Double-observer

On June 30 (between 9 a.m. and 11 a.m.) and July 4 (between 4 p.m. and 6 p.m.), 2015, we conducted one session of DDO and one session of IDO, respectively. For both methods we observed the animals from the same sectors and vantage points used for the mark-resight (Fig. 1a). During the IDO, two observers (MB–observer A, FZ–observer B) independently surveyed the same sector, at the same time, recording the number of animals observed and their respective locations. At the end of the 4 surveys, data were compared to determine the number of individuals seen by observer A and not seen by observer B and vice-versa, and the number of individuals seen by both observers (Williams et al., 2002). During the DDO, in each of the 4 sectors the two observers (MB, FZ) were alternatively designated as "primary" and "secondary" observer. During a given survey, the primary observer notified the secondary one of each detected marmot. The secondary observer then recorded the number of animals detected by the primary observer, as well as those recorded by himself (Williams et al., 2002). The same sampling scheme was repeated for each of the 4 surveyed sectors, switching the roles of primary and secondary observer. For both IDO and DDO, observations of kids were discarded to avoid bias in the estimate, and data were analysed using the software DOBSERV (Nichols et al., 2000).

Tab. 1 shows a summary of the different methodologies used in this study.

Results

Capture-mark-recapture

During the 8 sessions of capture-mark-recapture we had a total of 62 capture events with 39 individually marked animals. Details of capture-recapture events are appended in Tab. S1. Tab. 2 reports the results of the model selection applied to the 8 models of Otis et al. (1978): 4 models had $\Delta AICc<7$ (M_0, M_b, M_h, M_{bh}) and were thus retained to perform model averaging. The averaged parameters yielded an estimate of n=56 individuals with a CV of 17% (95% CI=45–87).

Table 2 – Results of the model selection on the 8 capture-mark-recapture models of Otis et al. (1978) (see text for details) fitted to investigate population size of Alpine marmot in the study site within the Stelvio National Park in the spring of 2015. The table reports values of Akaike's Information Criterion corrected for small sample size (AICc), differences in AICc ($\Delta AICc$) between each model and the model with the lowest AICc, the Akaike's weights (Weight), number of parameters (Num. par.) and deviance. Selected models are shown in bold.

Model	AICc	$\Delta AICc$	Weight	Num. par.	Deviance
M_0	**86.545**	**0.000**	**0.634**	**2**	**57.512**
M_b	**88.561**	**2.017**	**0.231**	**3**	**57.490**
M_h	**90.636**	**4.092**	**0.082**	**4**	**57.512**
M_{bh}	**92.679**	**6.135**	**0.029**	**5**	**57.490**
M_t	94.376	7.831	0.013	9	50.786
M_{tb}	95.315	8.770	0.008	10	49.590
M_{th}	98.659	12.115	0.001	11	50.786
M_{tbh}	99.627	13.083	0.001	12	49.590

Table 3 – Results of the model selection on the 6 Poisson log-normal mark-resight models (see text for details) fitted to investigate population size of Alpine marmot in the study site within the Stelvio National Park in the spring of 2015. The table reports values of Akaike's Information Criterion corrected for small sample size (AICc), differences in AICc (ΔAICc) between each model and the model with the lowest AICc, the Akaike's weights (Weight), number of parameters (Num. par.) and deviance. Selected models are shown in bold.

Model	AICc	ΔAICc	Weight	Num. par.	Deviance
$\alpha(.)\sigma(0)$	**154.269**	**0.000**	**0.508**	**2**	**149.945**
$\alpha(.)\sigma(.)$	**155.112**	**0.843**	**0.333**	**3**	**148.446**
$\alpha(cov)\sigma(0)$	**158.039**	**3.770**	**0.077**	**4**	**148.896**
$\alpha(.)\sigma(cov)$	**159.378**	**5.109**	**0.040**	**5**	**147.613**
$\alpha(cov)\sigma(.)$	**159.425**	**5.155**	**0.039**	**5**	**147.660**
$\alpha(cov)\sigma(cov)$	164.735	10.466	0.003	7	147.235

Mark-resight

During the 6 sessions of mark-resight we observed 108 marked individuals (79 marked identified, 29 marked unidentified) and 65 unmarked individuals. Details of resighting occasions are appended in Tab. S2. The Bowden's estimator yielded an estimate n=62 individuals and a CV of 6.4% (95% CI=54–71). Because one crucial assumption of BOWE is that marks must be quite evenly distributed among groups (Fattorini et al., 2007), we performed a Spearman's rank correlation test between group size and number of marked individuals within each group: the correlation was positive and highly significant (rho=0.49, $p<0.001$), thus confirming the fulfillment of the assumption. Tab. 3 reports the results of the model selection conducted using the Poisson log-normal estimator: 5 out of 6 models had ΔAICc<7 and were thus retained to perform model averaging. The averaged parameters yielded an estimate of n=62 individuals with a CV of 5.3% (95% CI=55–69).
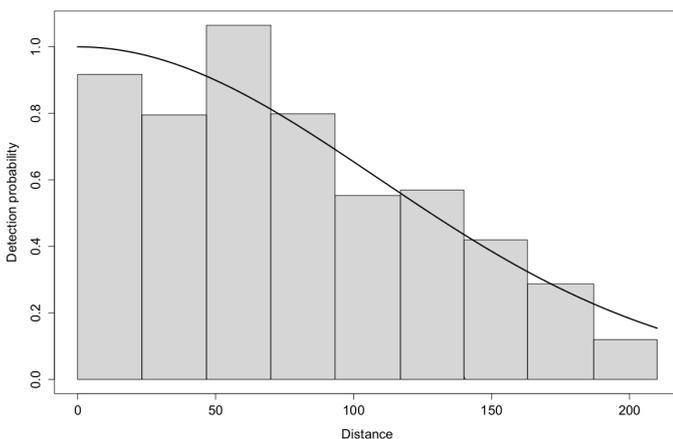
Figure 2 – Detection function giving rise to the best fitting of the observed distances, obtained using a half normal key function without series adjustment, used to estimate the population size of Alpine marmot within the Stelvio National Park with line transect sampling in the summer of 2015.

Line transect distance sampling

On the whole, we collected 198 observations (n=269 marmots), with an average encounter rate of 7.74 animal/km. Among the tested models, a half normal model without adjustment terms and without covariates was the best one in terms of AICc (Fig. 2, Tab. 4). The estimate based on this model gave an abundance of 24 marmots, with confidence intervals ranging from 19 to 31 individuals. When considering covariates in the estimation of the detection function, none of the models we considered had a lower AICc than the half normal model without covariates.

Double observer

In the two sessions of double-observer, we observed 21 individuals and 15 individuals for the IDO and DDO, respectively. Details of sighting trials are appended in Tab. S3. The software DOBSERV returned an estimate of n=24 individuals with a CV of 12% (95% CI=22–35) for

the IDO, while for the DDO it yielded an estimate of n=15 individuals with a CV of 5% (95% CI=15–20).

Fig. 3 shows a summary of the population size estimates obtained with the different methodologies. The minimum number of individuals alive (MNA=54) was obtained by adding the maximum number of unmarked marmots observed during one session of mark-resight (n=15) to the 39 marked individuals. We used the t-test to perform pairwise comparisons of the estimates: the difference between the BOWE estimate and the CMR estimate was not significant (two-sided t-test: $p=0.57$), while the difference between BOWE and DS estimates was highly significant (one-sided t-test: $p<0.01$), as well as the difference between CMR and DS (one-sided t-test: $p<0.01$). The difference between the DS estimate and the IDO estimate was highly not significant (two-sided t-test: $p=1$).

Discussion

Despite the lack of information about the real population size, useful indications can be obtained from the comparison between the minimum number of individuals alive in the population and the abundance estimates. The probabilistic approaches based on a subset of marked individuals (CMR, BOWE) were the only ones to include the MNA within their confidence interval, whereas the distance sampling approach and the double-observer approach underestimated population size, as the upper limits of their confidence interval did not include the MNA. While we acknowledge that in the period between MR and DS data collection some mortality events might have occurred, owing to predation by the golden eagle (*Aquila chrysaëtos*) or the red fox (*Vulpes vulpes*), we believe that the difference in the estimates obtained with the different methods largely reflects the violation of some assumptions underlying DS and DO.

The methods based on mark and recapture (or resighting) of individuals are often considered to be the gold standard for the estimation of population size, if the underlying assumptions are met (Pierce et al., 2012). We believe that the closure assumption was met in this study, as the 8 sessions of capture-recapture were conducted over a very short timeframe (8 days), during which events of mortality, immigration or emigration could be assumed negligible. Natality events were not a problem, since the kids were discarded from all analyses. Likewise, we believe that the assumption of permanence of marks was also met, given the short timeframe and the resighting of about 90% of marked individuals during all the survey sessions.

Similar results, but with higher precision (possibly owing to the greater number of encounter events), were obtained using a MR approach, as the two-sided t-test proved the difference between the CMR estimate and the MR estimates not significant. All the assumptions
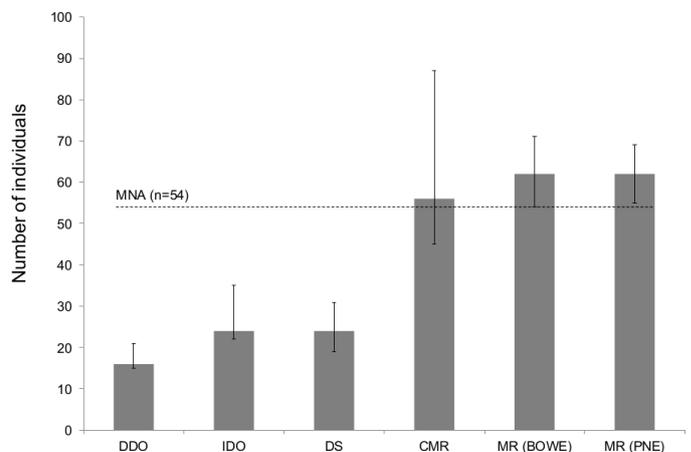
Figure 3 – MNA (horizontal dashed line) and size estimates of the marmot population in the study site within the Stelvio National Park obtained using different methods (capture-mark-recapture - CMR; mark-resight with Bowden's estimator - MR(BOWE); Poisson log-normal estimator - MR(PNE); line transect distance sampling - DS; dependent double-observer - DDO; independent double-observer - IDO) in the spring-summer of 2015. Vertical lines represent 95% confidence interval.

Table 4 – Distance sampling models fitted to estimate Alpine marmot abundance in the study site within the Stelvio National Park in the summer of 2015. The table reports information about used distance engine (Engine: CDS=conventional distance sampling; MCDS=multiple covariate distance sampling), key function (Key: HN=half normal; HR=hazard rate; Uni=uniform), covariates, adjustment terms (Adj.: -=no adjustment selected; Cos=cosine), effective strip width (ESW), significance of Kolmogorov-Smirnov goodness of fit test (K-S P); abundance estimation (N) with 95% confidence interval (95% CI); coefficient of variation (%CV). The selected model is shown in bold.

Engine	Key	Covariates	Adj.	Num. par.	ΔAICc	ESW	K-S P	N	95% CI	%CV
CDS	Uni	-	Cos	2	0.38	144.1	0.854	22	(16–29)	15.0
		-	-	1	0.88	146.3	0.597	21	(17–26)	10.6
	HN	**-**	**-**	**1**	**0.00**	**130.3**	**0.335**	**24**	**(19–31)**	**12.2**
	HR	-	-	2	1.06	143.7	0.769	22	(17–28)	12.4
MCDS	HN	Operator	-	2	1.29	130.1	0.697	24	(19–30)	11.2
		Disturbance	-	2	1.39	130.1	0.286	24	(19–30)	11.2
		Weather	-	2	1.79	130.3	0.323	24	(19–30)	11.2
		Habitat	-	2	1.50	130.2	0.420	24	(19–30)	11.2
	HR	Operator	-	3	3.09	146.8	0.909	21	(17–27)	11.0
		Disturbance	-	3	3.12	146.8	0.909	21	(17–27)	11.0
		Weather	-	3	3.16	147.0	0.909	21	(17–27)	11.0
		Habitat	-	3	3.12	147.1	0.909	21	(17–27)	11.0

underlying the Bowden's estimator were met in this study: beside confirming the demographic closure and the permanence of marks (see above), the Spearman's test showed a significant, positive correlation between the number of marked individuals and group size, thus supporting the even distribution of marks among groups (Fattorini et al., 2007). The Poisson log-normal estimator yielded very similar results to BOWE, but with a narrower confidence interval. Because PNE assumes independently and identically distributed resighting probabilities for each animal (McClintock et al., 2009), we suspect that the tendency of marmots to form groups and defend territories may have caused a slight violation of such assumption both in space (when a group is detected, several animals are likely to be detected) and in time (territorial animals are likely to be spotted in their territories over different trials). This lack of independence may entail a contagion among resightings, eventually leading to narrower confidence intervals (Fattorini et al., 2007).

The estimate obtained with line transect distance sampling was apparently biased low. Our study was the first attempt to use this method for estimating Alpine marmot abundance and density. In general, the line transect distance sampling approach can be difficult to apply in mountainous habitats because of the natural roughness of the landscape. For the Alpine marmot, in particular, the eco-ethology of the species most likely leads to the violation of some fundamental assumptions of the method. Three crucial assumptions are reported by Buckland et al. (2001), and highlighted by Thomas et al. (2010): a) objects on the line or point are detected with certainty, b) objects do not move prior to detection and c) measuring is exact. We are confident that our survey and analysis respected b), because the particular features of our study area, i.e. open grasslands without particular obstacles, simple orography and good visibility, allowed us to detect animals even from far distances and thus before any movement due to our presence. We respected c) as well, thanks to the use of accurate and precise tools that allowed us to take exact distances. In addition to the non-random distribution of the line transects, assumption a) was the most difficult to respect and our underestimate of marmot density may be also ascribed to its violation. Theoretically, marmots on the transect line should be easy to detect, so that their detection probability should be 1 at zero distance and it should decrease with increasing distance, and it may possibly be affected by other covariates. However, marmots display burrowing behaviour, thus the assumption of seeing everything at distance zero was probably not satisfied as some animals may hide below the transect. More generally, detection probability of marmots appears to be conditional on the burrowing habit of the species, which reduces the number of animals available for detection at any given distance.

Nichols et al. (2000) suggested that DDO is likely to hold promise when detection probability is >0.40. The observations conducted during the mark-resight survey yielded detection probabilities of marked marmots of about 0.68, on average. Although this value is greater than that suggested by Nichols et al. (2000), the DDO returned a severe un-derestimate of population size: a successful application of this method on the target species would likely require higher detection probabilities, especially given the high variation of sightability owing to the impact of daily meteorological conditions on the animals' activity. The IDO performed relatively better, though the estimates remained well below the minimum number of individuals alive in the population. Some assumptions underlying the IDO are difficult to meet, notably the assumption that each survey should generate a simple random sample of the entire population (Magnusson et al., 1978; Suryawanshi et al., 2012). In highly social species with territorial behaviour, such as the Alpine marmot, sighting independence in time and among animals rarely holds in real situations (Fattorini et al., 2007). Further, as noted by Suryawanshi et al. (2012), the detection probabilities of groups greatly depend on the activity of the animals, the distance of the group from the observer and the topographical characteristics of the landscape. To allow the modelling of individual heterogeneity, we suggest it would be worth investigating the use of IDO with multiple (e.g. 4) observers, and analyse data in a fashion similar to that used for the CMR approach (Williams et al., 2002). Nonetheless, IDO models estimate the conditional probability of detection, i.e. the probability of detection given that animals are available for detection: the semifossorial habits of the target species is thus likely to hamper the performance of this method, regardless of the number of observers.

Our results suggest that the CMR and the MR approach may constitute robust alternatives to estimate population size of Alpine marmots. Because the main drawbacks of such methodologies are clearly associated with the capturing effort, when the aim is to obtain reliable estimates of abundance we suggest that MR, rather than CMR may be employed, as in several circumstances it may reduce the costs associated to fieldwork (McClintock and White, 2012). Furthermore, the computation of MR estimates using Bowden's estimator in NOREMARK is much easier than building CMR models in MARK, and it can be easily done even by non-expert users. When the interest is in the large-scale, long-term monitoring of changes in population size, however, both the CMR and the MR methods appear unsustainable.

From a management standpoint, the use of methods that do not rely on marked individuals — such as distance sampling or double-observer — would be desirable. Both methods have benefits and drawbacks: DS may be somewhat easier to carry out in the field, as it appears operatively simpler and it requires only one observer, but it is more time-consuming and computationally more demanding than the DO methods. Furthermore, the assumption of perfect detection of objects on the line or point, as well as the conditional probability of detection need to be accounted for. While the point transect distance sampling may reduce the impact of the operator on the detection probability (Pelliccioli and Ferrari, 2013), issues related to the burrowing behaviour are more difficult to address. Generally, imperfect detection in DS is commonly encountered, for example, in surveys of marine mammals, since they are frequently below the sea surface. This issue can be taken into ac-

count by estimating a multiplier to correct the probability of presence of the animals as a function of the operator disturbance effect, but also with respect to ecology and behaviour of the target species (e.g. burrowing habits), for example using radio-tagged animals. ✍

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Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Table S1 Sex-age class and capture history of the marmots.

Table S2 Sex-age class, number of observations for each marked individual, unidentified and unmarked individuals.

Table S3 Number of observed individuals during the double-observer sessions.