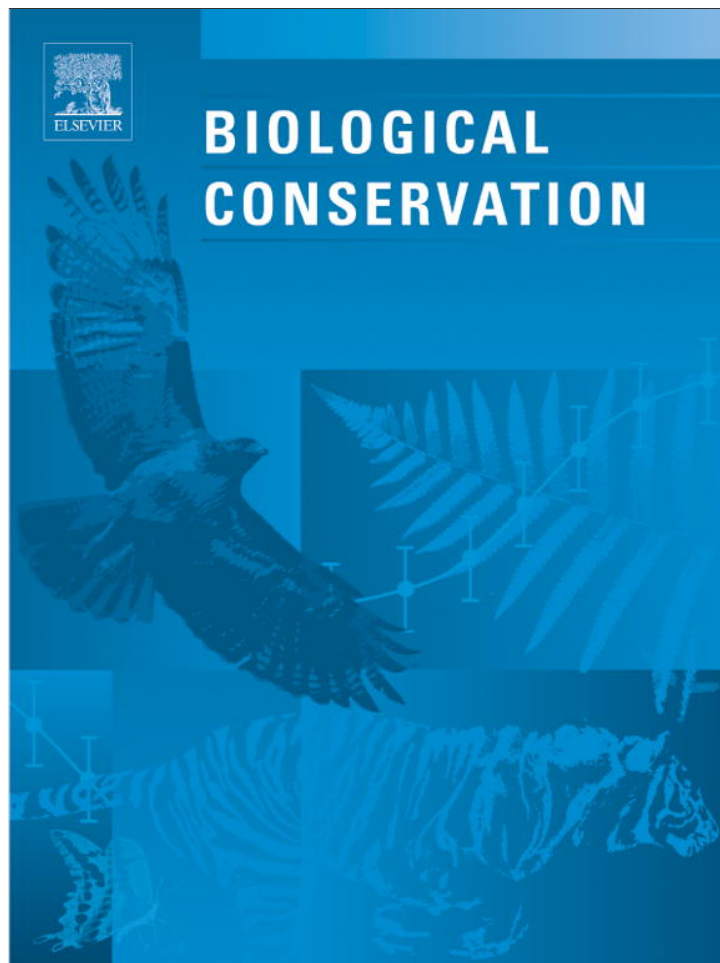


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## A multiple data source approach to improve abundance estimates of small populations: The brown bear in the Apennines, Italy

Vincenzo Gervasi<sup>a,\*</sup>, Paolo Ciucci<sup>a</sup>, John Boulanger<sup>b</sup>, Ettore Randi<sup>c</sup>, Luigi Boitani<sup>a</sup>

<sup>a</sup>Dipartimento di Biologia e Biotecnologie, Università di Roma "La Sapienza", Viale dell'Università 32, 00185 Roma, Italy

<sup>b</sup>Integrated Ecological Research, 924 Innes, Nelson, BC, Canada V1L 5T2

<sup>c</sup>Laboratorio di Genetica, Istituto Superiore per la Protezione e la Ricerca Ambientale, Via Ca' Fornacetta 9, 40064 Ozzano Emilia, Bologna, Italy

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### ABSTRACT

When dealing with small populations of elusive species, capture–recapture methods suffer from sampling and analytical limitations, making abundance assessment particularly challenging. We present an empirical and theoretical evaluation of multiple data source sampling as a flexible and effective way to improve the performance of capture–recapture models for abundance estimation of small populations. We integrated three data sources to estimate the size of the relict Apennine brown bear (*Ursus arctos marsicanus*) population in central Italy, and supported our results with simulations to assess the robustness of multiple data source capture–recapture models to violations of main assumptions. During May–August 2008, we non-invasively sampled bears using systematic hair traps on a grid of 41 5 × 5 km cells, moving trap locations between five sampling sessions. We also live-trapped, ear-tagged, and genotyped 17 bears (2004–2008), and integrated resights of marked bears and family units (July–September 2008) into a multiple data source capture–recapture dataset. Population size was estimated at 40 (95% CI = 37–52) bears, with a corresponding closure-corrected density of 32 bears/1000 km<sup>2</sup> (95% CI = 28–36). Given the average capture probability we obtained with all data sources combined ( $\hat{p} = 0.311$ ), simulations suggested that the expected degree of correlation among data sources did not seriously affect model performance, with expected level of bias <5%. Our results refine previous simulation work on larger populations, cautioning on the combined effect of lack of independence and low capture probability in application of multiple data source sampling to very small populations ( $N < 100$ ).

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### 1. Introduction

Population size and density are critical parameters in the decision-making processes for management and conservation of animal populations (Williams et al., 2002). They play a key role in determining genetic diversity, susceptibility to stochastic mortality factors, and ultimately population extinction risk (Beissinger, 2002). A reliable assessment and monitoring of population size over time also contributes to evaluate the effectiveness of conservation strategies, and subsequent population responses (Sutherland et al., 2004).

Besides accuracy, high precision is an important requisite of population size estimates, especially when dealing with small populations threatened by extinction. In such cases, estimates with a large associated uncertainty, although unbiased, retain a limited value in a conservation perspective, as it can be difficult and

controversial to detect trends over time (Yoccoz et al., 2001). Accordingly, the extra effort (and costs) necessary to enhance precision should allow increased reliability of trend estimates, and hence evaluation of past and current conservation actions.

Capture–recapture (CR) models currently represent a widely used analytical framework for the estimation of demographic traits in wildlife populations. By analysing individual encounter histories, derived from repeated sampling of the population under study, CR models allow the estimation of a great variety of demographic and life history traits, such as population size (Cubaynes et al., 2010), survival rate (Lebreton et al., 1992), recruitment (Pradel, 1996), population trend (Pradel, 1996), dispersal, and access to reproduction (Pradel et al., 1997).

However, the application of CR models to rare or elusive species poses particularly daunting challenges, both on practical and theoretical grounds. Surveying elusive species is often jeopardized by the low effectiveness of survey methods, as a consequence of the species' behaviour, activity, preferred habitats, and low overall densities (McDonald, 2004). Both rare and elusive species therefore often risk of being sampled with low capture probability, which results in a small proportion of the whole population being sampled.

\* Corresponding author. Tel.: +39 0649914750; fax: +39 06491135.

E-mail addresses: [vincenzo.gervasi@nina.no](mailto:vincenzo.gervasi@nina.no) (V. Gervasi), [paolo.ciucci@uniroma1.it](mailto:paolo.ciucci@uniroma1.it) (P. Ciucci), [boulange@ecological.bc.ca](mailto:boulange@ecological.bc.ca) (J. Boulanger), [ettore.randi@isprambiente.it](mailto:ettore.randi@isprambiente.it) (E. Randi), [luigi.boitani@uniroma1.it](mailto:luigi.boitani@uniroma1.it) (L. Boitani).

These conditions make the estimation of demographic parameters particularly challenging, as statistical power and precision of CR estimators is directly related to sample size and capture probability (Cohen, 1988). Despite these problems, a great number of demographic studies are focused on rare and elusive species, as they are often poorly known or threatened by extinction (McDonald, 2004).

During the last decade, a large variety of non-invasive sampling tools have become available for the demographic study of species with low detectability. Among these, non-invasive genetic sampling (Taberlet et al., 1996) and photo-trapping (Kucera and Barrett, 2011) largely prevail, as they usually do not require previous live-trapping and marking of individual animals in the population. These recently introduced sampling techniques have been usually applied as stand-alone methods to produce individual encounter histories needed for CR modelling (Karanth et al., 2004; Bellemain et al., 2005). Nevertheless, this approach is expected to provide small sample sizes, when dealing with rare or elusive species, which can result in low analytical power and low precision of estimates (White et al., 1982; Boulanger et al., 2008; Proctor et al., 2010).

An interesting and practical alternative is the adoption of multiple data sources in CR-based population assessments to confront low sample sizes and biases with single data sources with resulting gains in accuracy and precision (Boulanger et al., 2008). Such an approach has been since successfully applied to demographic studies of brown bears both in North America (Boulanger et al., 2008; Kendall et al., 2008, 2009) and Europe (Gervasi et al., 2008), and humpback whales (*Megaptera novaeangliae*; Madon et al., 2011), but never to populations smaller than 250 individuals.

The Apennine brown bear (*Ursus arctos marsicanus*) is a typical example of a terrestrial carnivore population in urgent need for a reliable estimation of its main demographic parameters, but also exhibiting all the challenging sampling and modelling issues of rare and elusive species. This population is the last remnant, genetically isolated portion of a formerly larger one, historically distributed along a wider range along the central Apennines in Italy (Ciucci and Boitani, 2008). According to a 2004 preliminary estimate, far less than 100 bears comprise the core of this population (Gervasi et al., 2008), currently distributed in the Abruzzo, Lazio and Molise National Park (PNALM) and its outer buffering area (about 1300 km<sup>2</sup>). Despite its endangered status and high conservation value, the implementation of an information-based management policy on the Apennine brown bear has been strongly limited by the lack of a reliable estimation of its remnant abundance (Ciucci and Boitani, 2008). Accordingly, the lack of a formal monitoring program over the last 20–30 years has prevented a reliable estimation of population trend, and hence the assessment of (i) what factors mainly threaten the population; (ii) its chances of persistence over the next decades, and (iii) the effectiveness of past and current conservation actions (Ciucci and Boitani, 2008).

To address these problems, a preliminary DNA-based population estimate was attempted in 2004 (Gervasi et al., 2008), which had low precision due to low capture probabilities combined with high and unmodeled heterogeneity. In order to overcome these problems, we successively carried out a pilot study in 2007 to enhance field procedures and evaluate the estimates resulting from the improved hair-snag sampling success (Gervasi et al., 2010). Given the small size of this bear population, the pilot study results suggested that hair-snag sampling alone would have likely corresponded to inadequate capture probabilities for adequate estimate precision (Gervasi et al., 2010).

In this paper we illustrate how integrating multiple data sources allowed us to increase sample size and capture probability, thereby enhancing the performance of CR models. Our main objectives were to integrate non-invasive genetic sampling, live-trap-

ping and resightings of marked bears into a multiple data source framework, and apply closed population CR models (Huggins, 1991) to produce a precise estimate of the Apennine brown bear population size and density. We also compared the performance of the full versus reduced sampling designs to empirically evaluate the additional benefits of using concurrent sampling techniques. Finally, we performed simulations to explore how sample size and capture probability can affect the robustness of multiple data source CR models to violations of their main assumption, namely correlation among different data types, and correlation among individuals in the study population. Being based on a relict brown bear population, our application provides an empirical example of how the concurrent use of several sampling techniques can help overcome the main limitations in estimating the abundance of small populations of rare and elusive species, thus further refining the results of previous simulation work based on larger population sizes (Boulanger et al., 2008).

## 2. Materials and methods

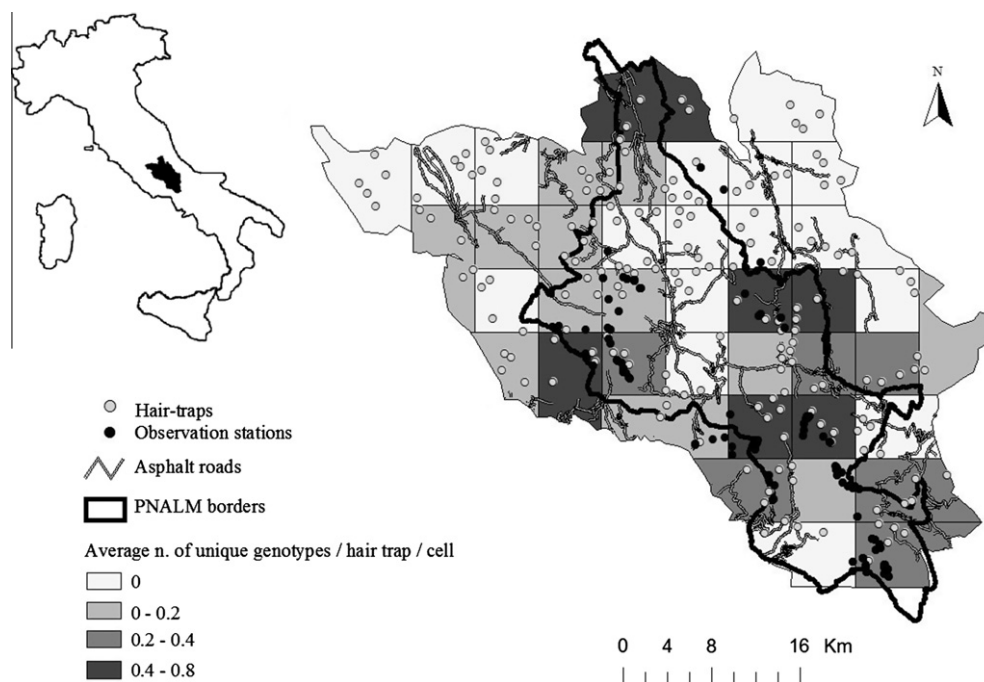
### 2.1. Study area

The Abruzzo, Lazio and Molise National Park (PNALM) crosses the Apennines mountain chain in southern-central Italy, encompassing 507 km<sup>2</sup> of core protected area and 787 km<sup>2</sup> of external buffer area (Gervasi et al., 2010). The landscape is primarily a mosaic of largely continuous mountain forest and a limited number of small human settlements, mainly located in the valley bottoms. Vegetation is characterized by extensive deciduous forests, mainly with beech *Fagus sylvatica* and oak *Quercus* spp. Mountain tops often exceed 2000 m a.s.l., and timberline is usually located at 1800–1900 m. Almost 30% of the study area is above timberline, characterized by bare rocks, alpine meadows and a patchily, highly concentrated distribution of buckthorn (*Rhamnus alpina*) aggregations, whose berries are an important food source for bears during late summer. Even though geographic and ecological connections between the PNALM and other mountain and forested areas of central Italy are still potentially available, the persistence of medium to high human-induced mortality risks in the peripheral part of the bear range still prevents a significant expansion and geographical recovery of the species (Falcucci et al., 2009).

### 2.2. Sampling methods

We used three independent sampling methods in order to increase sample size and coverage: non-invasive genetic sampling through hair-snagging, live-capture, and sightings of marked bears.

We collected bear hair samples following standard procedures (Woods et al., 1999), by using baited traps systematically distributed according to a 1187 km<sup>2</sup> grid of 41 5 × 5 km cells (Fig. 1). Between May 14th and August 3rd, 2008, we activated one hair trap in each cell for five sequential sampling sessions of 12 days each, moving traps (≥ 1 km) between successive sessions to reduce the probability of behavioural responses of previously hair-snagged bears (Boulanger et al., 2006). Based on the guidelines provided by our previous experience with hair-snag sampling in the specific conditions of our study area (Gervasi et al., 2008, 2010), we also used Geographic Information System (GIS) derived suitability values (Falcucci et al., 2009) to aid the identification of trap site locations. All suitable sites were also field inspected by experienced personnel before the actual survey. Hair traps consisted of a 25–30 m perimeter of barbed wire around 4–7 large trees, at the centre of which we piled stones and tree branches covered with moss, where we poured 5–6 L of rotten blood and fish as a scent lure (Woods et al., 1999).



**Fig. 1.** Hair-snag sampling grid and observation stations used to estimate the abundance of the Apennine brown bear population in the Abruzzo, Lazio and Molise National Park (PNALM; Italy, May–July 2008). Different grey levels indicate the average number of individual bears sampled in each session.

We checked traps at the end of each session and classified each collected sample based on the number of hairs, to differentially assess the performance of DNA extraction (class 1: <5 hairs/sample; class 2: 6–10 hairs/sample; class 3: >10 hairs/sample). Hair samples macroscopically belonging to non-target species were discarded at hair collection, whereas we examined doubtful samples using microscopic hair features (Teerink, 2004).

During 2004–2008, as part of a broader study on the ecology of the Apennine bear population, we live-trapped bears of all age classes, and instrumented them with coloured ear-tags and/or VHF/GPS collars (adults only). We also drew blood samples to assess their multi-locus genotype (see below). Similarly to Kendall et al. (2008), we included in the CR dataset all live-trapped bears known to be still present and available in the study area during the 2008 hair-snag sampling period, thereby increasing the overall sample size. In addition, as marked bears constituted a sub-sample of visually recognizable bears, we also used resights of radio-collared bears, including females with cubs, as an additional sampling method. Because bears are usually difficult to observe in our study area, we carried out systematic observations at buckthorn (*R. alpina*) patches, where bears congregate in summer (Tosoni, 2010). The sighting effort, structured in three consecutive 2–3 day sessions (28 July–27 September 2008; 21–40 vantage points), was concentrated at dawn and dusk in the core of PNALM, where buckthorn patches are distributed at higher densities (Fig. 1). Collars and/or ear-tags on instrumented bears were only used to visually recognize individual bears, and telemetry was not used to facilitate their observation. With the exception of family units (i.e., females with cubs, see below), only sightings of marked bears and family units with at least one marked individual were recorded, excluding all observations involving doubtful individual identification. Moreover, as is customary for unduplicated counts of females with cubs, identification of unmarked family units was conservatively based on their temporal and spatial distances compared to reference values (Ordiz et al., 2007; Tosoni, 2010). Also in this case, unmarked female bears with cubs were excluded from subsequent analyses, as it was impossible to individually identify them and distinguish

them from genetically sampled bears. The overall structure of the sampling design is illustrated in Fig. 2.

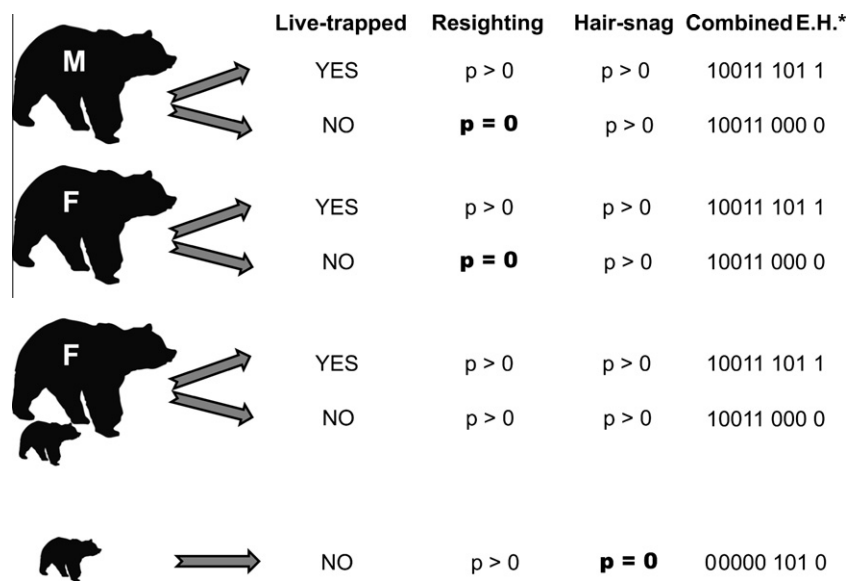
Such a sampling design, with both observable and unobservable adults bears, and with different identification criteria for marked and unmarked females with cubs, was in accordance with the fundamental assumption of multiple data source modelling, which requires all individuals in the population to be granted a non-zero capture probability with at least one (but not all) sampling technique (Boulanger et al., 2008). Multiple data source sampling, therefore, strongly relaxes capture probability assumptions for the additional sampling methods, so that an incomplete sampling coverage with one or more of these data sources is not an analytical issue (Boulanger et al., 2008). In our case, as hair snagging was based on a systematic effort across the entire study area, similarly to other applications on brown bears (Boulanger et al., 2008; Kendall et al., 2008, 2009), we expected hair snagging to provide all adult bears with such baseline capture probability (see Fig. 2). Accordingly, the additional sampling techniques, although focusing on a more specific segment of the bear population, were not expected to cause any relevant bias in population estimation.

Based on previous hair-snagging attempts carried out in 2007 (Gervasi et al., 2010), we did not expect to hair-snag the cubs of the year in the spring, likely due to their small body size in this period compared to the height of the barbed wire. Including resight data of family units was therefore instrumental not only to enhance estimation of capture probability for the adult segment of the population, but also to complement coverage of other sampling methods (i.e., hair-snagging).

### 2.3. Genetic methods

We analyzed hair samples following standard protocols for low DNA quality and quantity samples (Paetkau, 2003; Taberlet et al., 1996). We sexed all samples using the Amelogenin AMG gene, and obtained individual genotypes through replicated PCRs at eleven autosomal microsatellite loci (MU51, G1D, G10B, G10C, MU59, MU50, MU05, MU11, G10L, MU15, G10P).





**Fig. 2.** Structure of the multiple data source sampling design used to compose individual encounter histories (EHs) to estimate the abundance of the Apennine brown bear population in the Abruzzo, Lazio and Molise National Park (PNALM; Italy, May–July 2008). For each bear belonging to the categories we considered (adult males, adult females without cubs, adult females with cubs, cubs of the year) the individual encounter history (exemplified in the IV column) would be determined by the outcome of hair-snagging (sessions 1–5), resightings (sessions 6–8) and previous live-trapping (session 9). Data sources for which a certain bear category had null capture probability are highlighted in bold. \*The combined encounter history shows a possible sequence of sampling events for each bear group. An “all zero” sequence for a specific sampling technique refers to a null capture probability.

We followed a multiple-tube procedure, consisting of four successive steps: (1) we screened and amplified all samples 4 times at 3 loci (MU15, MU50, AMG), removing all samples which showed <50% positive PCRs; (2) we further processed the remaining samples to obtain 4 additional PCR replicates at all 11 loci; (3) we produced a reliability score  $R$  for each multilocus genotype, using RELIOTYPE (Miller et al., 2002) and removed all samples with  $R < 0.95$ ; (4) we identified consensus genotypes, using GIMLET V.133 (Valière, 2002), including an allele if it was scored at least twice.

We also applied a set of post-process quality controls, following recommendations by Paetkau (2003). In particular, we repeated the genotyping process for the following cases: (1) all genotypes identified through a single hair sample; (2) all single and double mismatch pairs of individuals; (3) all samples attributed to the same genotype, but collected at remarkable geographic distances; (4) all samples attributed to radiocollared bears whose sampling locations were inconsistent with the estimated individual home range. We estimated per genotype allelic dropout (ADO) rates as the ratio between the number of single-locus genotypes including at least one allelic mismatch, and the number of replicated single-locus genotypes, whereas false alleles (FAs) were defined as those detected only once in at least 7 replicated PCRs (Taberlet et al., 1996). We also calculated the probability of identity ( $P_{id}$ ) and the full siblings probability of identity ( $P_{sib}$ ), to evaluate the power of our markers in correctly discriminating between different genotypes (Waits, 2001).

#### 2.4. Population estimation and density

We used Huggins closed population models (Huggins, 1991) in Program MARK (White and Burnham, 1999) for the estimation of the Apennine brown bear population size. As a first step, we combined data from the three sampling methods described above to construct individual encounter histories. For each sampled bear, we recorded hair-snag captures in sessions 1–5, resights in sessions 6–8, and residency status of previously marked bears in session 9. This approach was allowed for closed capture models, in

which the relative order of sessions is irrelevant to parameters estimation, unless any behavioural response is present in the data (Boulanger et al., 2008). We assumed our hair-snag data to be free from any behavioural response, because we moved hair traps between successive sessions, providing no reward to sampled bears. In addition, bears were sighted when spontaneously aggregating at buckthorn patches, and their sightings were therefore not influenced by previous hair-snag sampling nor live-capture events. In addition, although buckthorn berries constitute a highly rewarding food source for bears, they are naturally available in the study area and their probability of being used by bears is independent from our observation effort. In a CR modelling context, this implies that resighting rates of a marked bear at a buckthorn aggregation were not affected by previous observations.

We constructed candidate models for the hair-snag sampling using variables such as sex, age class, time variation, and linear trend (T) (Boulanger et al., 2004; Kendall et al., 2008). To model the effect of geographic closure violation on capture probability, we also included, as an individual covariate, the linear, quadratic and log-transformed distance of mean sampling location to the edge of the sampling grid (DTE). For resights of individually recognizable bears, we defined models including sex and two broad age classes: cubs (<1 year) and adults (>1 year). We used a binary covariate to separate bears with collars and/or eartags from those that were not visually recognizable, thus assuring that these latter were assigned a null capture probability for the observation sessions. We defined temporal covariates for sighting probabilities as total observation effort, expressed as the total number of observation stations used in each session, and also considered models with linear trends in sighting probabilities. To account for expectedly lower sighting probabilities farther from the observation areas (Kendall et al., 2008), we also included the linear, quadratic and log-transformed distance of the mean sampling location to the closest vantage point as an individual covariate. Finally, we used an intercept function to model capture probability for the live-capture session, thus obtaining the proportion of live-trapped bears in the overall sample, as an estimate of live-capture probability (Kendall et al., 2008).

We assessed relative model support using the sample size adjusted Akaike's Information Criterion ( $AIC_c$ ) of model fit. The model with the lowest value of the  $AIC_c$  was considered to be the most parsimonious (Burnham and Anderson, 2002). To account for the degree of uncertainty in model selection, we modelled averaged parameters estimates using the Akaike weights as an index of their relative support (Burnham and Anderson, 2002). We calculated 95% log-based confidence intervals of model averaged population size estimates, accounting for the minimum number of bears in the study area during the sampling period ( $M_{t+1}$ ; White et al., 2002).

We estimated bear density accounting for the degree of violation of the geographic closure due to temporary emigration from the sampling grid, using a recently developed regression-based method (Ivan, 2011) implemented in program MARK. For each of the GPS-collared bears on the study area during summer 2008, we calculated the proportion of GPS locations within the sampling grid during the hair-snag sampling period ( $\bar{P}$ ), and included it as an individual covariate in the Huggins analysis. We then used logistic regression to compare the relative support of models estimating  $\bar{P}$  as a function of the linear, quadratic, and log-transformed distance of each bear from the sampling grid (DTE), and models with constant  $\bar{P}$  across the study area (Ivan, 2011). We also tested for differences in  $\bar{P}$  between male and female bears. The main advantage of this method, compared to previous procedures (White and Shenk, 2001) is that it is less sensitive to the assumption that radio-collared bears have the same spatial distribution as the DNA bears, thus providing unbiased estimates of bear fidelity (and hence of density) when live-trapping and radio-tracking effort are not evenly distributed in the study area, which is very often the case in radio-tracking studies. Finally, to obtain an indication of density variation inside the sampling grid, we also considered the average number of individual bears sampled inside each cell (Kendall et al., 2008). This provided us with a preliminary indication about the partitioning of bear density inside the study area, introducing a future formal occupancy analysis (McKenzie et al., 2002) for the Apennine brown bear at PNALM.

In order to evaluate the efficiency of our sampling design (i.e., dataset derived from all three sampling methods) compared to simplified (less expensive) ones, we repeated model selection and population size estimation using two simplified designs, one derived from hair-snag data only, the other combining data from hair-snag and live-trapping. This allowed us to compare each of the three designs in terms of point estimates, precision, and average capture probability, thus empirically evaluating their relative performance.

### 2.5. Robustness to assumption violations

We performed two sets of Monte Carlo simulations using R (v. 2.11.0, R Development Core Team, 2008) to explore the robustness of the Huggins estimator to violations of its main assumptions, namely a correlation in capture probability between: (a) different data sources, and (b) different individuals within a single data source.

As re-sightings and individual recognition require bears to have been previously live-trapped, our modelling design was potentially affected by a violation of the independence assumption between these two data sources. Therefore, we generated a virtual population of the same size and sex/age structure as the estimated one (see Section 3). Then, we sampled the population using a nine-session multiple data source sampling design, similarly to our real dataset, and simulated two different sampling scenarios, performing 1000 iterations for each set of simulated parameters. In the first scenario, we kept the probability of an individual bear to be live-trapped and to be subsequently resighted completely independent.

In the second scenario, to simulate the actual sampling conditions of our design, we modelled sighting probability of adult bears conditional on a previous live-trapping event. Both scenarios were run under increasing levels of the average capture probability associated with each data source (range: 0.1–0.5). For each iteration, we analyzed simulated encounter histories in Program MARK by using the most supported model in our previous Huggins analysis (Model 1 in Table 2), and compared results of the two scenarios in terms of accuracy, precision, and confidence interval coverage of the population size estimates.

We also ran a second set of simulations to assess the effect of potential correlation in capture probability among individuals using the same sampling technique. In particular, cubs of the year sighting probabilities cannot be assumed to be independent from those of their mothers, as they are tightly associated in this time of year; furthermore, we based individual recognition of litters conditional on the identification of marked mothers, so that the family unit, rather than individual bears, was the actual sampling unit. Using the same virtual population as above, we contrasted two simulation scenarios. First, mothers and cubs of the year were sampled as separate individuals, each of them with an independent capture probability; second, mothers and cubs were sampled as a unique sampling unit, thus more realistically reflecting our sampling conditions. Also in this case, we simulated both scenarios with increasing levels of the sighting probability (range 0.1–0.5), and additionally evaluated the effect of increasing proportions of females with cubs of the year within the overall population (range: 0–60%).

## 3. Results

### 3.1. Sampling

During the five sessions of hair-snag sampling, we activated and checked 205 sites. We collected bear hair at 19% of them. Out of a total of 466 samples, 260 were attributed to non-target species (see Table S1 in Supporting Information). The remaining 206 were all genetically confirmed to be bear samples, 55 of which were included into class 1, 36 into class 2, and 115 into class 3. Out of them, 76 samples (38%) provided <50% positive PCR after the screening step. In the remaining 130 samples, genotyping success was extremely different for the 3 hair abundance classes: 22% were successfully genotyped in class 1, 67% in class 2, 82% in class 3. The number of hairs in a sample also affected genotyping error rates, whose average value were  $ADO = 0.10$  and  $FA = 0.01$ .  $P_{id}$  and  $P_{sib}$  were  $1.76 \times 10^{-5}$  and  $4.43 \times 10^{-3}$ , respectively.

Successfully genotyped samples belonged to 20 unique bears (10 F and 10 M). Out of them, 10 bears were sampled in one session, 5 in two sessions, 4 in three sessions, whereas one bear was sampled in all the five sessions. Nineteen of these bears had already been detected through genetic sampling in previous years, and were therefore >1 year old at the time of sampling in 2008. The remaining genotype coincided with a previously live-trapped adult male bear (Table S2). Therefore, as expected, our 2008 genetic sampling did not include any cub of the year.

During the hair-snag sampling period, 17 marked bears (8 males and 9 females) were still alive and available in the study area, and were therefore included in the CR encounter history matrix (Table S3). Of these, we individually resighted and recognized 11 adult bears (7 F and 4 M). During the observation sessions we also identified 6 family units, comprising 10 cubs of the year. Four of these family units comprised marked females, for a total of 7 cubs, whereas 2 family units, for a total of 3 cubs, were unmarked and therefore distinguished based on spatio-temporal distances between their sightings (Table S3). As all genetically sampled bears were >1 year old, this allowed us to include the 10 cubs in the

**Table 1**

Model selection results for the Huggins closed population estimation from all data sources, applied to the 2008 sampling of the Apennine brown bear population in the PNALM, Italy. Abbreviations for the data sources indicate hair-snag (HS), resights (RESs), and live-trapping (LT). Parameter abbreviations indicate the number of observation stations (TOEs), the distance of each bear from the grid edge (DTE), and the distance of each bear from the closest observation area ( $l_{d_{obs}}$ ).

Model no.	Model	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	w <sub>i</sub>	No. par	Deviance
1	HS: $p(\text{sex} + \text{age} + t_4)$ RES: $p(\text{sex}) + \text{LT}$	332.76	0.00	0.138	8	316.31
2	HS: $p(\text{sex} + \text{age})$ RES: $p(\text{sex}) + \text{LT}$	332.98	0.22	0.123	7	318.64
3	HS: $p(\text{sex} + \text{age} + \text{DTE} + t_4)$ RES: $p(\text{sex}) + \text{LT}$	333.41	0.64	0.099	9	314.85
4	HS: $p(\text{sex} + \text{age} + T_f)$ RES: $p(\text{sex}) + \text{LT}$	333.47	0.71	0.096	8	317.03
5	HS: $p(\text{sex} + \text{age} + t_4)$ RES: $p(\text{sex} + T) + \text{LT}$	334.25	1.49	0.065	9	315.69
6	HS: $p(\text{sex} + \text{age} + t_4)$ RES: $p(\text{sex} + \text{TOE}) + \text{LT}$	334.48	1.71	0.058	9	315.92
7	HS: $p(\text{sex} + \text{age} + t_4)$ RES: $p(\text{sex} + l_{d_{obs}}) + \text{LT}$	334.59	1.83	0.055	9	316.03
8	HS: $p(\text{sex} + \text{age} + T)$ RES: $p(\text{sex}) + \text{LT}$	334.69	1.93	0.052	8	318.25
9	HS: $p(\text{sex} + \text{age} + t_4)$ RES: $p(\text{sex} + d_{obs}^2) + \text{LT}$	334.73	1.97	0.051	9	316.17
10	HS: $p(\text{sex} + \text{age} + \log(\text{DTE}) + t_4)$ RES: $p(\text{sex}) + \text{LT}$	334.75	1.99	0.051	9	316.19

overall encounter history with no risk of double-counting unique bears, whereas the 2 unmarked adult females, sighted as members of family units, were excluded from subsequent analyses, because we were not able to distinguish them from other genetically sampled female bears. Therefore, the structure of our dataset allowed us to distinguish cubs of the year vs. bears >1 year old into different groups for our subsequent CR-based population estimate.

### 3.2. Population estimation and density

Including all data sources simultaneously, 10 models in the Huggins analysis were equally supported by the data ( $\Delta AIC_c < 2$ ), and the most supported model (Model 1 in Table 1) had 13% of total weight. Hair-snag capture probability was mainly a function of sex and age class, with an additional decrease in capture probability during session 4. Less supported factors were linear trend and linear distance of mean sampling location from the grid edge. Males exhibited a higher average hair-snag probability ( $\hat{p} = 0.482$ , 95% CI = 0.334–0.642) than females ( $\hat{p} = 0.216$ , 95% CI = 0.128–0.338). The average proportion of live captured bears in the data set or adult bears was 0.459 (95% CI = 0.307–0.619), with no difference between females and males.

Sighting probabilities were primarily influenced by sex (Table 1), with marked female bears and cubs of the year exhibiting the same sampling probability ( $\hat{p} = 0.521$ , 95% CI = 0.386–0.654) on average higher than adult males ( $\hat{p} = 0.221$ , 95% CI = 0.102–0.413). Other variables influencing variation in the sighting probability included linear trend, total observation effort, and linear distance of mean sighting location from the closest vantage point (Table 2). The overall average capture probability, estimated by pooling all sex and age classes, and all sampling methods, was 0.311 (95% CI = 0.216–0.438).

By model averaging parameters estimates, we obtained a total population size of 40 bears (95% CI = 37–52), corresponding to 11 males (95% CI = 11–13), 18 females (95% CI = 16–24) and 11 cubs of the year (95% CI = 10–15), with an overall female-biased sex ratio of 1.386: 1 (95% CI = 1.331–1.518: 1).

**Table 2**

Model selection results for the closure violation analysis, applied to the 2008 sampling of the Apennine brown bear population in the PNALM, Italy. Abbreviations for the data sources indicate hair-snag (HS), resights (RES), and live-trapping (LT). Parameter abbreviations indicate the number of observation stations (TOE), the distance of each bear from the grid edge (DTE), the distance of each bear from the closest observation area ( $l_{d_{obs}}$ ), and bear fidelity to the sampling grid ( $\hat{P}$ ).

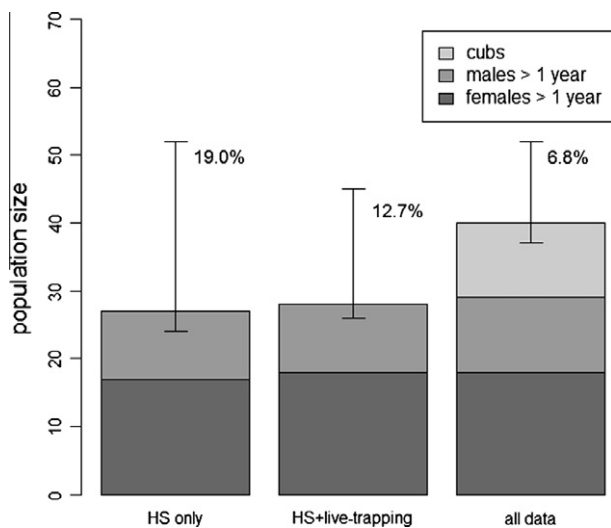
No.	Model	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	w <sub>i</sub>	No. par	Deviance
1	HS: $p(\text{sex} + \text{age} + t_4)$ RES: $p(\text{sex}) + \text{LT}$ ; $\hat{P}(\text{sex} + \text{DTE}^2)$	3010.76	0.00	0.57	9	2992.21
2	HS: $p(\text{sex} + \text{age} + t_4)$ RES: $p(\text{sex}) + \text{LT}$ ; $\hat{P}(\text{sex} + \text{DTE})$	3011.52	0.76	0.39	9	2992.97
3	HS: $p(\text{sex} + \text{age} + t_4)$ RES: $p(\text{sex}) + \text{LT}$ ; $\hat{P}(\text{sex} + \log \text{DTE})$	3017.01	6.25	0.02	9	3010.94
4	HS: $p(\text{sex} + \text{age} + t_4)$ RES: $p(\text{sex}) + \text{LT}$ ; $\hat{P}(\text{sex})$	3034.43	23.66	0.00	8	3017.99
5	HS: $p(\text{sex} + \text{age} + t_4)$ RES: $p(\text{sex}) + \text{LT}$ ; $\hat{P}(\cdot)$	3123.27	112.50	0.00	7	3108.93
6	HS: $p(\text{sex} + \text{age} + t_4)$ RES: $p(\text{sex}) + \text{LT}$ ; $\hat{P}(\text{DTE})$	3143.07	132.30	0.00	8	3014.64

Based on 9601 GPS locations of 13 radio-collared bears (7 F and 6 M), the analysis of bear fidelity to the sampling grid revealed that the proportion of time spent by each bear inside the sampling grid ( $\hat{P}$ ) was a function of bear sex, and of the individual distance from the grid edge (DTE). The most supported model (model 1 Table 2) included a quadratic effect of DTE on  $\hat{P}$ , with bear fidelity decreasing in the peripheral part of the study area. The best model revealed also a reduced fidelity of male bears, with respect to females. The sex-specific effect of DTE on bear fidelity to the sampling grid is shown in Fig. 4. Resulting from the model selection procedure, the average bear fidelity to the sampling grid was 95.1%, corresponding to a closure corrected density of 32 bears/1000 km<sup>2</sup> (95% CI = 28–36). Density inside the PNALM was 1.5 times higher than within its external buffer zone, but areas with a high density of bears were also located at the extreme periphery of the sampling grid (Fig. 1).

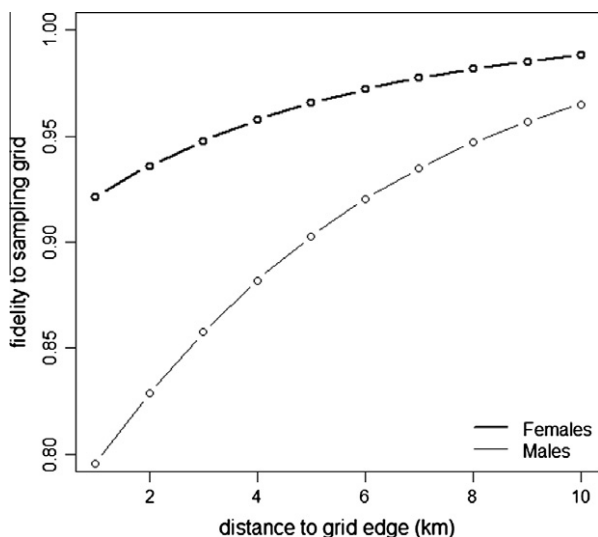
Estimates resulting from the HS-only and HS-livetrapping designs were 27 (95% CI = 27–54) and 28 (95% CI = 28–45) bears, respectively, therefore 30% and 27% lower than the estimate produced using all data sources simultaneously (Fig. 3). Out of this, the negative bias in the estimation of the adult segment of the population was 6.8% and 3.4%, respectively, whereas the remaining portion of bias (23.2% and 23.6%, respectively) was due to the inability by the reduced designs to include cubs into population estimates. The design including all data sources also corresponded to a much higher precision (CV = 6.8%) of the final estimate with respect to the simplified designs (CV = 19% and 12.7% for the HS-only and the HS-livetrapping designs, respectively; Fig. 3). Accordingly, the overall average capture probability increased from 0.25 to 0.31, proceeding from the simplest to the most integrated design.

### 3.3. Robustness to assumption violations

Both bias and loss of precision due to the correlation between live-trapping and sighting probabilities were strongly mediated by the average overall capture probability (Fig. 5). Low average



**Fig. 3.** Comparison of three alternative sampling designs in terms of sex and age – specific population size estimates for the Apennine brown bear in the Abruzzo, Lazio and Molise National Park (PNALM; Italy, May–July 2008). Error bars represent 95% confidence interval, percentages indicate the corresponding CV.



**Fig. 4.** Relationship between the proportion of time spent by each bear on the sampling grid during the 2008 hair-snag sampling at PNALM and the individual distance from the edge of the study area, as derived from Model 1 in Table 2. Separate curves for male and female bears are reported.

capture probability ( $p < 0.2$ ) caused negative significant bias (Fig. 5a), which was instead reduced to less than 3% when the overall average capture probability was  $> 0.3$  (Fig. 5b). Simulations also showed that confidence interval coverage was not affected by correlation between data sources, with values ranging around the nominal 95% interval coverage for all simulated scenarios.

A different pattern was observed when exploring the effect of correlation between resights of females and their cubs. In this case, lack of independence generated a minor reduction of confidence interval coverage in the estimation of both age classes, likely as an effect of variance underestimation (Fig. 6c). Nevertheless, such reduction never corresponded to a confidence interval coverage  $< 90\%$ . In addition, the extent of bias increased for an increasing proportion of females with cubs in the population, when simulating a resight-only sampling design (Fig. 7a). However, such a negative effect was buffered by the contribution of the other data

types when including the observation sessions into the overall multiple data source design, regardless of the proportion of females with cubs in the population (Fig. 7b).

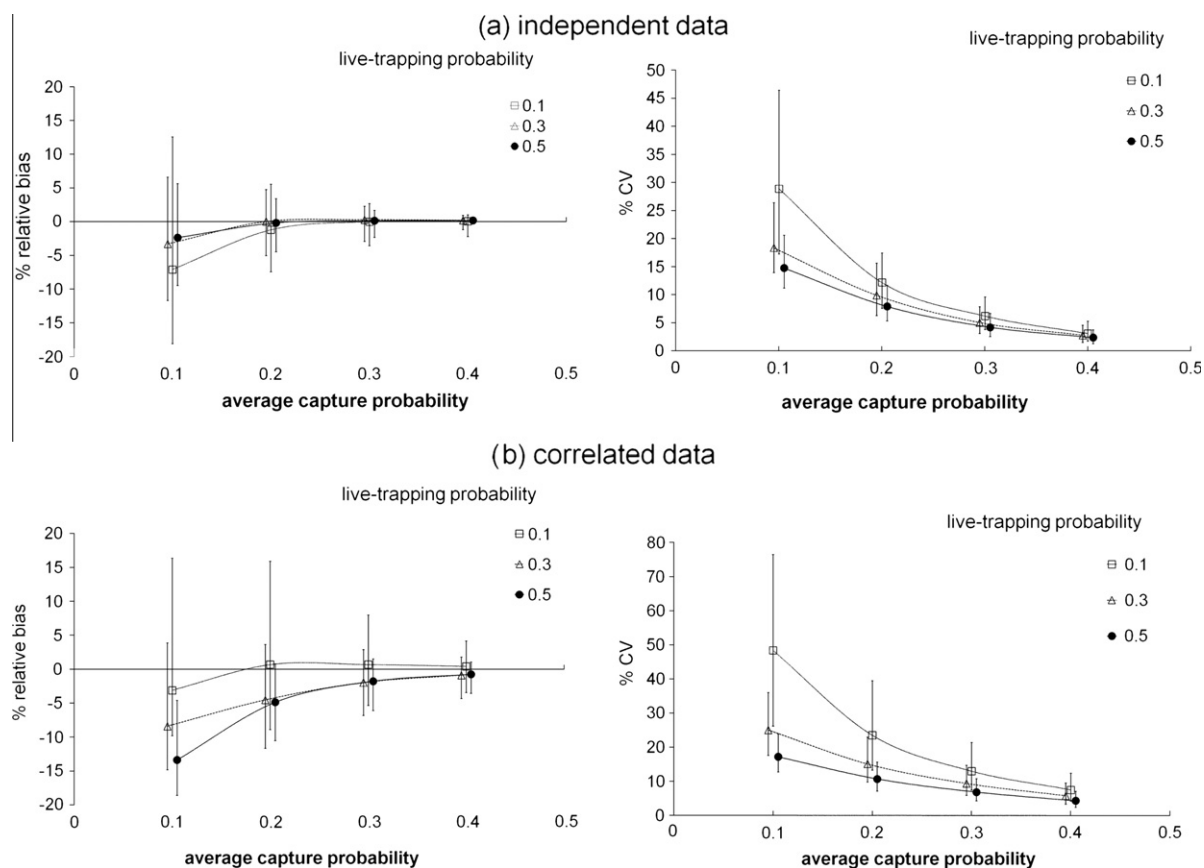
#### 4. Discussion

This study illustrates how multiple data source sampling can increase the viability of obtaining reliable demographic estimates for small populations. Although hair-snagging has been often applied as a stand-alone technique to estimate the size of bear populations (Proctor et al., 2010) the requirement of high capture probabilities to ensure adequate precision may limit its applicability to small populations. For example, an average capture probability  $> 0.4$  is required for a population of 50 bears to achieve an acceptable level of precision ( $CV < 0.2$ ) for management purposes (Proctor et al., 2010). Results of a previous pilot study in our study area (Gervasi et al., 2010) suggested hair-snagging alone would have likely not provided an adequate sample size to precisely estimate the expectedly small bear population size, due to expectedly low capture probabilities (0.14; Gervasi et al., 2010). By combining different sampling techniques into a multiple data source design, we managed to enhance model performance and increase estimate precision, corresponding to a 12% reduction in the coefficient of variation (from 19% based on hair-snagging alone to 7% using combined sampling methods; Fig. 3). Although lack of bias is an essential property of population size estimates, a precision high enough to allow meaningful assessment of population recovery or decline over time (Yoccoz et al., 2001) is a key aspect for conservation of endangered populations. For these reasons, although the benefits of the multiple data source approach are also apparent with larger populations (Boulanger et al., 2008), it is when dealing with small populations that an enhanced performance of CR models represents a particularly valid solution and provides direct benefits to conservation.

In our case, effectively integrating sub-optimal sampling conditions into a unique CR framework provided two important benefits compared to previous Apennine bear population estimates (Gervasi et al., 2008). First, by integrating DNA-based and resighting data, we were able to focus on different segments of the bear population, thus reducing the risk that a portion of the bear population remained undetected (cubs in our case). Whereas cubs are usually sampled using standard hair-snag protocols (Kendall et al., 2008; Proctor et al., 2010), they showed a close to zero hair-snag probability in our population (Fig. 2). This is supported by the evidence that no new genotypes were hair-snagged during the whole sampling period, as was expected based on a previous pilot study using hair-snag sampling in spring 2007, which also provided no genotypes from newborn cubs (Gervasi et al., 2010). It should be noted, however, that the snagging results we obtained in 2008 provided us a unique opportunity, in that cubs could be integrated and distinguished from adult bears in the sighting data set as no new genotypes were hair-snagged with respect to previous years. The null or very small probability of hair-snagging cubs during the spring in our bear population somehow contrasts with similar studies elsewhere (Kendall et al., 2008; Proctor et al., 2010), but provides additional evidence that age can be a strong source of heterogeneity in bear hair-snag based population estimation studies (Boulanger et al., 2004).

Second, the inclusion of sighting and live-trapping data also improved average capture probability for the adult (i.e.,  $\geq 1$  year) segment of the population. In fact, contribution from these two additional data sources made it possible to model the additional heterogeneity in capture probability, resulting from those marked individuals which remained virtually invisible to hair-snag sampling. This is an additional benefit of integrating concurrent data sources, as each contributes to enhance the estimation of capture



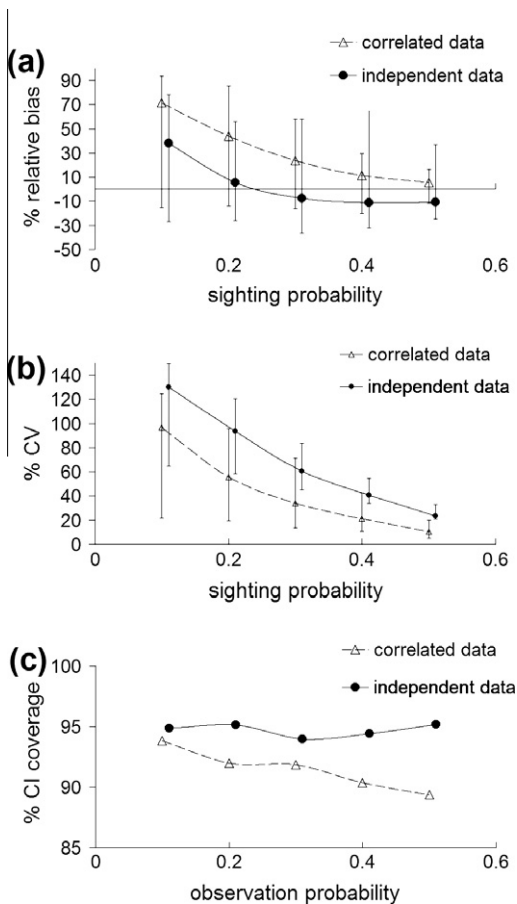


**Fig. 5.** Simulations of the Huggins estimator performance with an integrated sampling design using independent (a) vs. correlated (b) data sources. Bias and CV are shown as a function of the average individual capture probability and according to different levels of the live-trapping probability. Error bars represent 95% confidence intervals.

probability for all the other data types, with an overall positive feedback on the performances of CR estimators (Boulanger et al., 2008). In our specific case, the inclusion of live-trapping data provided a substantial benefit in terms of precision of the estimates, even though it did not cause a substantial change in point estimates, given the high average capture probability for the adult segment of the population. In addition, a comparison of the sampling designs that we adopted in 2004 vs. 2008 shows that, in our case, the multiple data source approach not only yielded higher precision of the final estimate but it was also more cost-effective. Costs of the 2004 survey totalled 198,674 € (including salaries, material, mileage and lab costs; Gervasi et al., 2008), and involved sampling at buckthorn patches which was not particularly efficient (62% of total survey costs). On the other hand, costs of non-invasive genetic sampling for the 2008 survey totalled 38,000 €, including salaries, material, mileage and lab costs. Although total costs would include also live-trapping and marking bears, as well as the costs of the systematic observation sessions, in our case these activities were part of a broader research project that was carried out independently from the actual population survey. Our application emphasizes the cost-effectiveness of an integrated data sources sampling design that allow creative combinations of systematic and opportunistic sampling methods. This can be a viable solution for small populations, especially when complementary data are being collected in the context of a broader population studies, or when opportunistic data are available through unconventional sources, such as public or hunters observations. Such an approach has been successfully implemented for the establishment of a long-term monitoring program of the Scandinavian brown bear population (Kindberg et al., 2009), where the combined use of non-invasive genetic sampling and opportunistic observations from bear

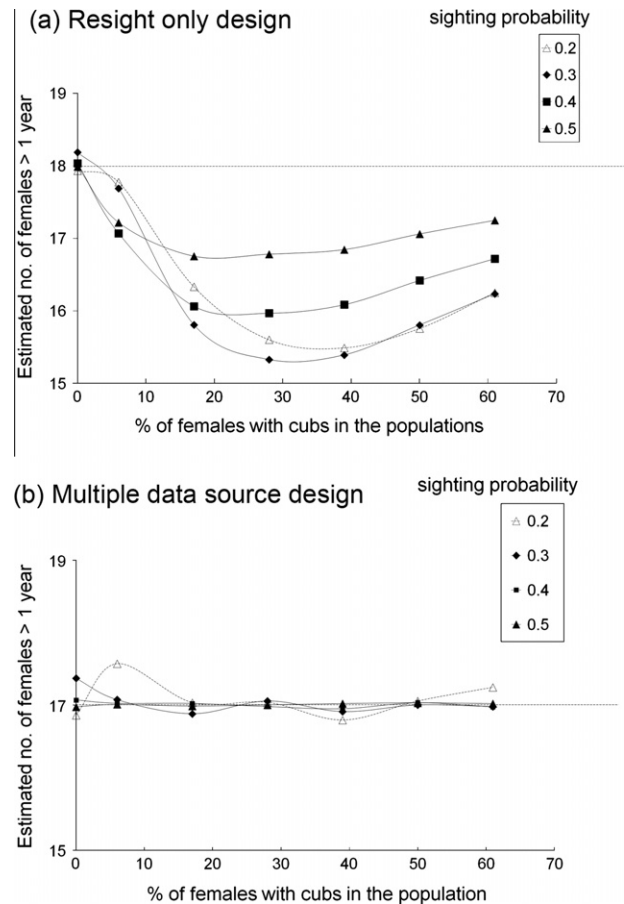
hunters is currently adopted as a standard sampling design. Similarly, De Barba et al. (2010) also assessed the cost-effectiveness of a combined sampling strategy, which comprised both systematic and opportunistic genetic sampling, for monitoring of the translocated brown bear population in the Western Alps. They showed that the opportunistic sampling, when coupled with other field methodologies, was an optimal additional sampling strategy to enhance the performance of CR estimators (De Barba et al., 2010).

To compare population estimates through time it is crucial that the same segments of the population are included in successive surveys, and for bear populations the cub component could be an issue. In this perspective, it should be noted that both the 2004 and 2008 surveys of the Apennine brown bear population included cubs: in 2008 because we integrated sighting data, including cubs, into a multiple data source CR framework; in 2004 because hair-snagging was performed in the fall, when cubs size makes them more susceptible to sampling (as evidenced by two new genotypes sampled together with an adult female with whom they shared at least one allele at each locus; Boulanger et al., 2004), and because systematic hair-snagging was substantially integrated with opportunistic hair-trap sampling at buckthorn patches, highly used by family units (Gervasi et al., 2008). Hence, a comparison between the 2004 (Gervasi et al., 2008) and 2008 point estimates (43 and 40 bears, respectively), suggests stability or a slight decrease (i.e., 1.7% per year) of the Apennine bear core population in the last 4-year period. Indeed, no significant improvements in conservation policy have taken place in these past years, nor lower levels of known mortality were reported for this population compared to previous decades (Ciucci P. pers. comm.). However, even though confidence interval for the 2004 and 2008 estimates totally overlap (95% CI = 35–67 and 37–52, respectively), low precision of the



**Fig. 6.** Simulations of the Huggins estimator performance with an integrated sampling design using correlated and independent observation probabilities of female bears with cubs. Bias (a), coefficient of variation (b) and confidence interval coverage (c) are shown as a function of sighting probability of family groups.

2004 estimate and its potential sources of bias make the above interpretation highly tentative. Still, the results of our population size assessment are instrumental in a conservation perspective as they allow us to better qualify and define three main fundamental aspects. First, they enable us to stress the high risk of extinction in the long term for this population, especially if viewed in conjunction with our 2004 estimate (Gervasi et al., 2008). In fact, based on our results, and on the sex and age structure of other non-hunted populations of brown bears (Wielgus, 2002), the number of reproductive females in the Apennine brown bear population is most likely 13 or lower, corresponding to about 30% of total population size. Wielgus (2002) found that a minimum of 54 reproductive females was required to ensure a low risk of extinction ( $p < 0.05$ ) within 20 years for the British Columbia brown bears. Accordingly, the brown bear population in the Cordillera Cantabrica of Spain, with an estimated 26 adult females, has been considered smaller than viable, as 41 adult females were minimally required to ensure persistence over 100 years according to locally observed mortality rates (Wiegand et al., 1998). Therefore, our results suggest that the Apennine brown bear population is likely well below the minimum threshold to be considered viable in the long term. Nevertheless, Sæther et al. (1998) estimated that a minimum of 6–8 adult female bears is sufficient to ensure a low ( $p < 0.1$ ) probability of extinction within 100 years, but their study was based on two expanding Scandinavian populations, with higher reproductive and survival rates, than those estimated elsewhere (Wiegand et al., 1998; Wielgus, 2002). While this provides some hope for our population, it also means that national and local



**Fig. 7.** Simulations of the accuracy of Huggins population size estimates under increasing proportions of adult females with accompanied cubs in the population. Model performance is also shown as a function of increasing sighting probability. (a) Resight-only sampling design, corresponding to three sessions of observations; (b) integrated sampling design, including five hair-snag, three observation and one live-trapping session. The dotted horizontal line indicates the correct simulated number of females in the population.

management authorities should do immediately their best as to curb human-caused mortality and to allow population growth. Second, the methodology we adopted allows for the establishment of a formal demographic monitoring of the population. The lack of such monitoring on this bear population did not allow up to now to evaluate population trends or the effectiveness of conservation measures (cf. Ciucci and Boitani, 2008). To this aim, the use of an integrated multiple sampling design seems to be the most cost-effective solution, not only to produce estimates of reasonable precision, but also to provide critical information on some demographic parameters. Third, the distribution of sampled bears in the study area (Fig. 1) showed that although relative bear density was 1.5 times higher inside PNALM borders than in the outer buffering area, in this area we also detected intense bear presence in this outer area. Hunting is allowed here and resource extraction is more flexible than inside the PNALM, so that a relevant proportion of the small bear population lives and finds key resources where it is supposedly more exposed to potential mortality causes (Ciucci and Boitani, 2008; Falcucci et al., 2009). Therefore, enhanced protection is urgently needed in these areas, where type and extent of human activities should be carefully planned and bear conservation should become a priority.

Our experience with the application of a multiple data source design to a very small population strongly supports the use of such an approach to overcome the limitations of dealing with only a few individuals in rare and elusive species. Nevertheless, before setting

up a multiple data source sampling design, particular caution should be devoted to the potential, combined effect of data correlation, both within and among the different data sources, and of low capture probability: especially in the case of small populations, these two factors can cause relevant bias and loss of precision. In fact, compared to the larger population sizes simulated by Boulanger et al. (2008), our simulations suggest that the effect of data correlation becomes progressively more serious at smaller population sizes and lower levels of the average capture probability. In particular, for a population of 200 bears, and using a multiple data source approach with complete correlation between two data sources, a capture probability  $>0.1$  has been shown to correspond to less than 5% bias in population size estimates (Boulanger et al., 2008). Our findings, on the other hand, reveal that the same simulation scenario with a population of 50 bears requires an average capture probability  $>0.3$  to ensure a similar level of bias in population estimates. It follows that, as CR closed population models exhibit reduced robustness to lack of independence between data sources at increasingly smaller sample size and lower capture probability (which are the main limitations affecting sampling of rare and elusive species), multiple data source surveys of very small populations ( $N < 100$ ) should be planned by carefully evaluating the trade-off between the limited performance of a single sampling method vs. the potential correlation among combined sampling methods.

In our specific case, simulations showed that despite partial correlation between data types (i.e., live-trapping and resighting) and individuals (i.e., females with cubs), no relevant bias in population estimation should be expected, mainly due to the average level of hair-snag and live-trapping probability that we reported. We also note that the increased precision provided by the integration of multiple data sources was not paid at the cost of an increased bias, or of reduced confidence interval coverage. This is a key aspect in the evaluation of sampling design performance, especially when applied to very small populations, as nothing worse can be produced, from a conservation perspective, than a biased abundance assessment providing a false sense of precision and reliability. Simulations clearly showed that this was not the case for our study. However, dealing with populations with lower detectability, correlation among data sources might produce different and more serious effects on the final estimates (Fig. 5). Correlation of mother-cub resights probabilities was somehow more problematic, but it effectively points out the trade-off nature inherent in the multiple data sources approach. In fact, on one hand the lack of independence between sighting probabilities of females and their cubs may potentially induce variance underestimation in CR population size estimates, and especially when a significant proportion of adult females has cubs (Fig. 7a); on the other hand the integration of resighting into a multiple data source design appears to strongly mitigate the negative effects of this type of correlation (Fig. 7b). It is worth emphasizing, however, that besides our specific application, robustness of the multiple data sources approach to data correlation cannot be regarded as a general rule. It should rather be evaluated on a case-by-case basis whenever conditional probability among different data sources is suspected, and according to both the expected degree of capture probability correlation among and within data sources, and the expected levels of capture probability.

Further generalizing from our specific case study, multiple data sources sampling can be an effective way to improve model performance for population estimation, especially when dealing with small populations for which a stand-alone sampling method is theoretically suspected to provide inadequate sample sizes and low capture probability. Besides the specific sampling design and data sources we used in our study, it should be emphasized that the most adequate sampling methods to be integrated into a multiple

data source framework are specific to the taxonomic groups under study and the conditions (e.g., area, logistics, habitat, accessibility) that characterize a given population survey. Whereas demographic studies of ursids have been traditionally based on hair-snag sampling (Boulanger et al. 2002; Gardner et al., 2010), non-invasive genetic surveys from scats are frequently used with canids (Cubaynes et al., 2010), and photo-trapping has progressively become the standard sampling procedure for felids and cetaceans (Karanth et al., 2004; Madon et al., 2011). The possibility of a joint application of these and other data sources, also in the analytical context of spatially explicit models (Royle and Young, 2008), has not been fully explored yet for these and other taxonomic groups. Their combination and evaluation into a multiple data source approach will require creative solutions to design surveys optimally adapted to local conditions. In the light of the recent availability of non-invasive sampling techniques, many of which can be applied to elusive and rare species (McDonald, 2004), we showed that a multiple data source framework, provided its basic assumptions are met, can substantially increase the opportunities to enhance precision of estimates of population abundance also in the case of particularly small populations.

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### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocon.2012.04.005>.

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