

Project LifeNAT/IT/000160 “ARCTOS” – Action E3



Ex post noninvasive survey of the core Apennine bear population (*Ursus arctos marsicanus*) in 2014



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Executive summary

From 26 May to 30 September 2014 we conducted the *ex post* non-invasive survey of the Apennine bear population in the core of its range (National Park of Abruzzo Lazio and Molise and immediately adjacent areas) in the central Apennines. Following an *ex-ante* and *ex-post* approach, our original aim was to obtain and compare reliable and precise population he estimates in 2011 and 2014 to detect trends in the population and directly assess the efficacy of the conservation actions implemented in the "Arctos" Life Project. Following the same sampling and modelling procedures developed and tested during the 2011 survey, in 2014 we used an exclusively noninvasive approach by integrating four sampling techniques (systematic hair snagging, rub tree sampling, opportunistic sampling at buckthorn patches, and sampling incidentally to other management activities). This was expected to enhance capture probability of individual bears and to increase sample size to be used in a capture-recapture closed population model (Huggins model in program MARK), and hence improve accuracy and precision of the final estimate. As it was done for the 2011 survey, all hair-traps used in 2014 have been dismantled by the end of the survey, and all the material used for sampling rub-trees and buckthorn patches has been accordingly removed from the field.

Similarly to the 2011 survey, samples collected in 2014 have been analysed by WGI (Wildlife Genetics International, B.C., Canada). According to the marker selection performed in 2011, we used for the 2014 samples 11 markers (plus sex), 9 of which in common with ISPRA, with the use of an additional marker in common to both labs (*G10P*) for equivocal cases. Conversion factors to translate WGI-scored into ISPRA-scored bear genotypes were reported in the 2011 report or are directly available from the authors of this report.

Overall, we collected 476 hair samples, ranging from 70 samples collected by incidental sampling to 207 by rub tree sampling. In total, 346 of the collected samples were analysed, the others being discarded as containing few and corrupted hairs. Of those analyzed, 276 samples proved positive to DNA extraction and multilocus genotyping, with an overall success rate of 79.8%. In total, we detected 44 different bears, corresponding to a sample sex ratio of 1:1.20 males to females, including cubs and management bears (individual multilocus genotypes are provided as an appendix to this report). Twenty-nine of these genotypes matched those of previously detected bears (i.e., bears who had been live-trapped and/or noninvasively sampled since 2000 in previous studies and surveys), but 15 bears were never detected before (either already present in the population during previous surveys but gone undetected, or newly added bears to the population after the 2011 survey). During the sampling period, one adult bear was shot and one cub died from unknown reasons, but they had not been included in our genotypes sample before they died.

The most supported AIC_c models included sampling methods and their interactions with sex, time, previous hair sampling history, and sampling effort by both rub tree and buckthorn sampling among the major drivers of capture heterogeneity. Accordingly, our final estimate of the population size was 50 (95% CI: 45 – 69, CV = 10.5%) bears, including cubs and management bears, corresponding to 22 (95% CI = 20-32) males and 28 (95% CI = 25-37) females. We estimated a population sex ratio of 1:1.27 MM:FF, and a closure-corrected density of 38.8 bears/1000 km². Crude estimates of associated population parameters, obtained integrating the estimate of population size with the indices of productivity obtained by unduplicated counts of females with cubs during 2011-2014, and by assuming that the population size remained stable across the 4-year period, include the mean proportion of cubs in the population (15.6%, 95%CI: 11 – 17%), and the mean proportion of reproductive females, both in the population (22.5%, 95%CI: 16 – 25%) and as a proportion of females of all ages (42.2%, 95%CI: 30 – 45%).

Compared to the point estimate of the population size in 2011, we definitively conclude that the Apennine bear population in its core range is not declining ($0.85 \leq \lambda \leq 1.14$) but is also not increasing. Instead, we revealed demographic stability despite the positive reproductive performance recorded annually from 2011 to 2014 (at least 31 cubs were born in the population, at an average of 7.8 cubs/year). A minimum of 12 bears were retrieved dead from unknown (n=3) and human-caused (poaching: n=3; vehicle accident: n=3; diseases: n=3) mortality during the span of the Life Arctos project. Excluding those that died in the peripheral portion of the range (n=2), minimum mortality levels in the PNALM ecosystem corresponded to 2.5 bears retrieved dead per year, a rate equal to that recorded in the years preceding the Life Arctos project, whereas minimum known levels of adult female mortality (1 adult female retrieved dead /year) exceeded those recorded in the preceding years (0.88 female bears/year). This (minimum known) mortality levels depress the inherent demographic capabilities of the core Apennine bear population to significantly expand its range beyond the core, forcing the population to remain at low numbers and at persistently high extinction risks. This is also showed by the lower mean heterozygosity and lower number of alleles that we observed in the new genotypes, assumed to represent a recent generation, which is suggestive of a very small effective population size (N_e) and the fast pace of genetic erosion. These findings once more highlight the urgency of immediate, effective, and aggressive conservation actions with the goal to allow for a rapid expansion of the range beyond the current core. Whereas the Life Arctos might have helped addressing bear-human conflicts and enhance bear-human coexistence locally, any of these best practices still need to be promoted and implemented over a much larger scale across the Apennines.

Riassunto

La fase *ex post* dell'azione E3 del progetto Life Arctos, coincidente con la stima di popolazione dell'orso bruno marsicano nella porzione del suo areale (Parco Nazionale d'Abruzzo Lazio e Molise e aree adiacenti) alla fine dell'ultimo anno di progetto (2014), è stata realizzata tra il 26 maggio e il 30 settembre. Successiva alla fase *ex ante*, condotta nell'estate del 2011, l'intento della fase *ex post* era permettere di rilevare eventuali tendenze nella popolazione di orso durante gli anni del progetto Life Arctos, al fine di valutarne l'efficacia direttamente in termini di dinamica di popolazione. In base al disegno di campionamento messo a punto nel 2011 (fase *ex ante*), anche nel 2014 sono state utilizzate tecniche di campionamento esclusivamente non invasive, integrando il campionamento sistematico sull'intera area di studio (hair snagging) con tecniche complementari (campionamento presso i 'rub tree', campionamento ai ramneti, e campionamento accidentale). Ciò ha permesso di aumentare la copertura campionaria della popolazione di orso e incrementare le probabilità di cattura individuali, entrambe condizioni necessarie per ottenere stime affidabili e precise tramite modelli di cattura-ricattura in popolazioni di così ridotte dimensioni (modello di Huggins implementato nel software MARK). Come avvenuto nel 2011, a conclusione del campionamento tutte le trappole per peli sono state smantellate e rimosse.

Le analisi genetiche sono state realizzate presso il WGI (Wildlife Genetics International, B.C. Canada), laboratorio specializzato nell'analisi di campioni non-invasivi di orso. In base a un insieme ideale di marcatori già selezionati nel 2011, sono stati utilizzati 11 marcatori (oltre al sesso) per l'individuazione dei genotipi individuali; 9 di questi marcatori sono in comune con il laboratorio di genetica dell'ISPRA, oltre a *G10P* anch'esso utilizzato in alcuni casi di difficile interpretazione confrontando genotipi recenti con quelli rilevati negli anni precedenti e analizzati da ISPRA.

In totale, sono stati raccolti 476 campioni di pelo, di cui 346 in quantità e qualità tali da essere utilizzati per le analisi genetiche. A loro volta, 276 di questi hanno portato all'individuazione di genotipi individuali affidabili, con un successo di tipizzazione del 79.8%. Sono stati quindi campionati in totale 44 orsi (i cui genotipi vengono riportati in appendice alla presente relazione) di cui 29 corrispondono a orsi già campionati negli anni precedenti e 15 ad orsi mai stati campionati prima. Due orsi, un maschio adulto e un cucciolo, sono stati trovati morti durante il periodo di campionamento, ma non erano stati campionati nelle precedenti settimane.

Ai fini della stima di popolazione, i modelli maggiormente supportati (AIC_c) individuano nelle tecniche di campionamento, nel sesso, nella progressione delle sessioni di campionamento, nella precedente storia individuale di campionamento, e nell'effettivo sforzo di campionamento sia ai rub tree che ai ramneti i principali fattori che influenzano l'eterogeneità di cattura. In base alla media di tali modelli, la stima finale di popolazione risulta essere di 50 orsi (IF 95%: 45 – 69 orsi), inclusivi di cuccioli e di orsi problematici e/o confidenti, comprendendo 22 (IF 95%: 20 – 32) maschi e 28 (IF 95%: 25 – 37) femmine. La stima corrisponde ad un rapporto sessi di 1:1.27 MM:FF e a una densità, corretta per la violazione dell'assunto della popolazione chiusa, di 38.8 orsi / 1000 km². Le stime di alcuni parametri demografici associati, ottenute assumendo che la popolazione sia rimasta costante dal 2011 al 2014, includono la proporzione di cuccioli nella popolazione (15.6%, IF 95%: 11 – 17%) e la proporzione media di femmine adulte rispetto sia all'intera popolazione (22.5%, IF 95%: 16 – 25%) che alle sole femmine di tutte le età (42.2%, IF 95%: 30 – 45%).

Confrontando la dimensione della popolazione di orso bruno marsicano a inizio e fine progetto Life Arctos (2011 e 2014), si può concludere che la popolazione nel suo areale centrale non è in fase di regressione ($0.85 \leq \lambda \leq 1.14$), né tuttavia in crescita. Al contrario, essa ha mostrato stabilità demografica, nonostante si sia registrata annualmente una discreta produttività (almeno 31 cuccioli nati dal 2011 al 2014, con una media di 7.8 cuccioli nati/anno). Nello stesso periodo di durata del progetto Life Arctos, 12 orsi sono stati recuperati morti a seguito di cause ignote (n=3), oppure direttamente o indirettamente determinate dall'uomo (bracconaggio: n=3; impatto con veicoli: n=3; malattie: n=3); escludendo quelli morti nelle porzioni periferiche dell'areale (n=2), un minimo di 2.5 orsi morti sono stati recuperati l'anno, ovvero 1 femmina adulta l'anno, con livelli di mortalità uguali o superiori a quelli rilevati negli anni antecedenti il progetto Life Arctos (0,88 femmine adulte/anno). Questi livelli di mortalità, da considerarsi minimi in quanto non si può assumere tutti gli orsi morti siano stati trovati o recuperati ufficialmente, deprimono le capacità intrinseche di ripresa della popolazione di orso bruno marsicano, determinando la persistenza di elevati rischi di estinzione legati alle dimensioni ridotte e alla distribuzione unica della popolazione. Ciò risulta anche evidente da una riduzione della eterozigosi media e del numero di alleli osservata nei nuovi genotipi campionati nel 2014 rispetto a quelli campionati precedentemente (≤ 2011), ad indicazione di elevati tassi di erosione genetica dovuti ad una popolazione effettiva (N_e) particolarmente ridotta. Questi risultati mettono ancora una volta in luce l'urgenza di azioni aggressive, urgenti e efficaci per facilitare la rapida espansione numerica e di areale della popolazione di orso bruno marsicano. A tal fine, laddove il progetto Life Arctos può avere individuato vie innovative per la risoluzione dei conflitti tra l'uomo e l'orso, le 'buone pratiche' per una migliore coesistenza con l'orso necessitano ancora di essere promosse e realizzate su vasta scala.

1. INTRODUCTION

The EU Life Natura project (LIFE+ NAT/IT/000160 "ARCTOS"), co-funded by the European Union and conducted by several national and regional conservation agencies, started in September 2010. The main aim of the project has been to promote several practical conservation actions in order to enhance bear conservation by improving long-term coexistence with humans. Within this project, the Department of Biology and Biotechnologies of the University of Rome "La Sapienza" has been responsible for the assessment of the bear population size in the PNALM at the first (2011) and last (2014) year of the project (Action E3), reflecting an *ex ante* – *ex post* population monitoring approach. Not only this facilitated an assessment of the efficacy of conservation interventions implemented during the project but, including previous population surveys conducted since 2004, has also provided for the first time the opportunity to assess population trends over a biologically meaningful stretch of time.

The Apennine brown bear, endemic to the central Apennines and believed by some to represent a subspecies (*Ursus arctos marsicanus*; Loy et al. 2008, Colangelo et al. 2012), is mostly relegated to a limited area centred around the Abruzzo Lazio and Molise National Park (PNALM), comprising a single population whose size and trends have been scarcely investigated prior to 2004 (Ciucci and Boitani 2008, Gervasi et al. 2008). A formal survey of this bear population was conducted for the first time in 2004 (Gervasi et al. 2008), but a more reliable assessment was carried out only in 2008 by means of noninvasive genetic sampling and a closed population modelling approach (Gervasi et al. 2012). Based on an integrated data-source approach (Boulanger et al. 2008), this method has been shown to provide reasonable accuracy for the PNALM bear population, notwithstanding the small population size and hence an expectedly small data set (Gervasi et al. 2008, 2012). Using the same general approach, in 2011, coincident with the *ex ante* phase of the Life Arctos E3 action, we further developed the survey strategy by uniquely using noninvasive sampling methods, considered more affordable and sustainable for the long-term monitoring of this bear population. In the same occasion, we also used the data we collected using all 4 sampling methods to empirically evaluate the efficiency of alternative (i.e., reduced) sampling strategies to be used in the future (Ciucci et al. 2013). According to these results, we decided to apply all noninvasive sampling methods for the 2014 survey, although attempting to reduce the redundancy of samples by any single sampling method.

Similarly to the 2011 survey, genetic analyses of the 2014 samples were conducted at Wildlife Genetics International (WGI, British Columbia, Canada), who previously determined conversion factors to allow translation of WGI- into ISPra-scores and vice versa (Paetkau 2012). Practical implications of these results for the consolidation of a comprehensive dataset have been already detailed elsewhere (Ciucci et al. 2012a). According to the marker selection procedures we empirically carried out in 2011, in 2014 we increased the efficiency of the marker system by using a total of 11 markers (plus gender), 10 of which in common with the ISPra lab. Therefore, 2 of the previously used loci by WGI (*MSUT-2* and *G10X*) were dropped in 2014. In addition, an additional marker in common with ISPra (*G10P*) was only used to compare equivocal cases (i.e., 1-3 MM pairs) when comparing new genotypes with those detected prior to 2011.

In addition to noninvasive genetic sampling, from 2011 to 2014 we also conducted each year unduplicated counts of females with cubs (Knight et al. 1995, Keating et al. 2002) to aid interpretation of estimates of population size and trends. Results of FWC counts from 2011 to 2014 have been already reported (Ciucci et al. 2011a, 2012, Tosoni et al. 2013, 2014). We finally collected all reliable information on bears reported dead during the past 4 years to be used as a figure of minimum known mortality.

Based on the results we obtained, the aims of this report are:

- to illustrate the 2014 sampling and modelling results, and to report the population size estimate we obtained in 2014;

- to compare population sizes of the Apennine bear population estimated in 2011 vs. 2014. Coupled with annual counts of reproductive females, and with minimum known mortality figures referred to the same time period, we accordingly interpret the observed trends in the bear population over these past 4 years;
- to report additional genetic data (i.e., multilocus genotypes) obtained through the intensive 2014 sampling within the core distribution of the Apennine brown bear. This contributes to the overall genetic database of the Apennine brown bears, useful to assess status and trends on a larger spatial and temporal scale across the central Apennines.

Although intermediate reports of the 2014 sampling progress have been already disseminated by the end of each hair-snag sampling session, this is a comprehensive report of the 2014 survey, including an overall assessment of population trends over the Life Arctos project and hence its immediate conservation responses.

2. METHODS

Following previous empirical and theoretical indications (Boulanger et al. 2008, Gervasi et al. 2010, 2012, Ciucci et al. 2013), in 2014 we aimed to compose individual encounter histories through a combination of sampling methods: namely, hair-snagging, rub-tree sampling, opportunistic sampling at buckthorn patches, and incidental sampling. In 2014 we used the same DNA-based CR modeling approach adopted in 2011, trying to reduce sample redundancy, especially for rub-tree sampling (see below). Similarly to 2011, we drafted a field protocol providing field work and implementation details for each sampling method to be circulated among field operators. Sampling strategies and corresponding field methods are succinctly illustrated below, although reference is often made to the more exhaustive report of the 2011 survey (Ciucci et al. 2013).

2.1 Preliminary activities and communication

Preliminary activities and communication within the survey team have been carefully planned and anticipated, as they were deemed critical to enhance success of the 2014 survey (Tab. 1).

Activity	Date	Administrations
Planning and logistical meetings	5, 17 Feb; 3 Mar	PNALM; CFS;BBCD
Development of a reference working schedule	11, 29 May	PNALM; CFS;BBCD
Motivational workshop	21 Mar	PNALM; CFS;BBCD
Planning workshop	12, 22, 23, 28 May	PNALM; CFS;BBCD
Assemblage of barbed wire and lure for single-trap use	9-25 May	PNALM; CFS;BBCD
Hair-traps field verification and marking	9 Apr-29 May	PNALM; CFS;BBCD
New rub-trees searching	1 Apr-31 May	PNALM; CFS;BBCD
Rub-tree arming with barbed wire	22 Apr-2 Jun	PNALM; CFS;BBCD
Meetings and contacts to organize helicopter flights	10 Apr-23 May	PNALM; CFS;BBCD
Helicopter flight to transport hair sampling material in inaccessible sites	6 Jun	PNALM; CFS;BBCD
Workshops to updating on sampling progress	16 Jun, 17 Ago	PNALM; CFS;BBCD
Assembling hair-traps at buckthorn patched	1-10 Ago	PNALM; CFS;BBCD
Dismantling hair-traps at buckthorn patches	27-30 Sep	PNALM; CFS;BBCD
Dismantling rub-trees	1-30 Oct	PNALM; BBCD
Helicopter flight to collect sampling material from inaccessible sites	14 Oct	PNALM; CFS;BBCD

Table 1. – Preliminary activities conducted in due time to allow proper sampling for the noninvasive survey of the core Apennine bear population in 2014.

In particular, from February through May 2014, we: (1) run several planning and organizational meetings with the chiefs responsible of the various administrations involved in the survey; (2) held professional and motivational workshops with field operators; (3) carried out preliminary field-work activities. The latter included: (a) field-verifying and marking each of 215 hair-traps systematically distributed across the survey area (April 9 – May 29); (b) field-verifying and pre-arming rub-trees already used in the 2011 survey (April 22 – June 2); (c) searching for new rub-trees, especially in areas where these were largely unrepresented in the 2011 survey (April 1 – May 31); (d) field-verifying buckthorn patches to be subsequently used in the survey; (e) preparing all materials needed to run the survey (e.g., lure, barbed wire, pegs; November 2013 – April 2014), and (f) translocation on site all material necessary for hair-snagging and buckthorn sampling, including 2 helicopter flights in most inaccessible areas.

2.2 Sampling strategies and field methods

To survey the Apennine brown bear population in the PNALM ecosystem, we adopted multiple sampling strategies including systematic hair-snagging, rub-tree sampling, opportunistic sampling at buckthorn patches, and incidental sampling (Gervasi et al. 2008, Ciucci et al. 2013) from May 26 to September 30, 2014. Hair-snagging ranged 8 weeks, from May 26 to July 26, while the other sampling methods extended through September.

Regardless of the sampling method, we considered a hair sample as a tuft of hairs entangled in one set of barbs (Woods et al. 1999). We collected each sample possibly containing guard hairs with bulbs with sterilized surgical forceps, and placed each sample in a paper envelope labeled with a uniquely numbered barcode. We then passed a flame under the barbs to remove any trace of hair to avoid contamination between sessions (Kendall et al. 2009). Paper envelopes containing samples were then stored in a dark place within a box with silica gel to avoid DNA degradation.

During sampling, but particularly for rub tree sampling, we occasionally collected ≥ 1 samples believed to be left by the same bear in a single sample occasion based on their proximity on the barbed wire (i.e., 'replicated' samples), especially during rub-tree sampling. In order to reduce costs of genetic analyses, differently than in 2011 in 2014 we limited collection of rub-tree samples per sampling occasion to one or maximum 3 among the best samples. Some of these replicates were sent to the lab for genetic analyses to provide additional source of DNA in case the primary sample did not yield a reliable genotype. Upon sample collection we discarded on the field all hair samples of other species, and used microscopic characteristics (Teerink 1991) to distinguish less obvious cases. Therefore, only macro- and microscopically pre-selected bear samples have been considered for genetic analysis.

2.2.1 Hair-snagging

We adopted systematic hair-snagging using 5x5 km grid cells covering the entire core area and 5 sampling sessions of 12 days each, and moved traps within each cell to increase trapping efficiency and to reduce the risk of behavioral responses. Number of cells and criteria for trap locations were the same we adopted in the 2011 survey. Overall, we surveyed 43 cells for a total of 215 traps in an area of 1221 km² (Fig. 1). Each trap consisted of 18-35 m of single-strand barbed wire encircling 5-11 trees and set at 50 cm from the ground (Woods et al. 1999). All hair traps were dismantled at the end of each session and moved to the new trap location.

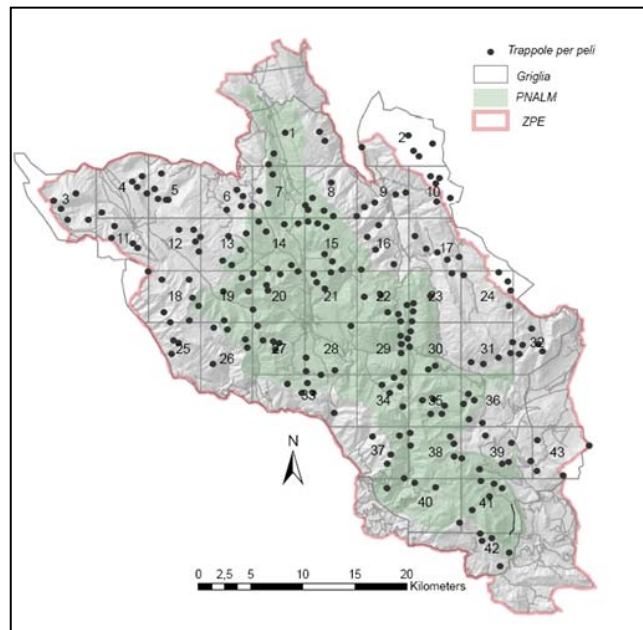


Figure 1. – Hair-snag (HS) sampling grid adopted for the survey of the Apennine bear population in the PNALM area (June – July 2014). In total, 43 sampling grids (5x5 km each) have been used, with some peripheral grids ≥ 25 km². Five sampling sessions of 12 days each were used, and hair-trap locations were moved between successive sessions for a total of 5 traps/cell and 215 traps (black dots) for the entire survey.

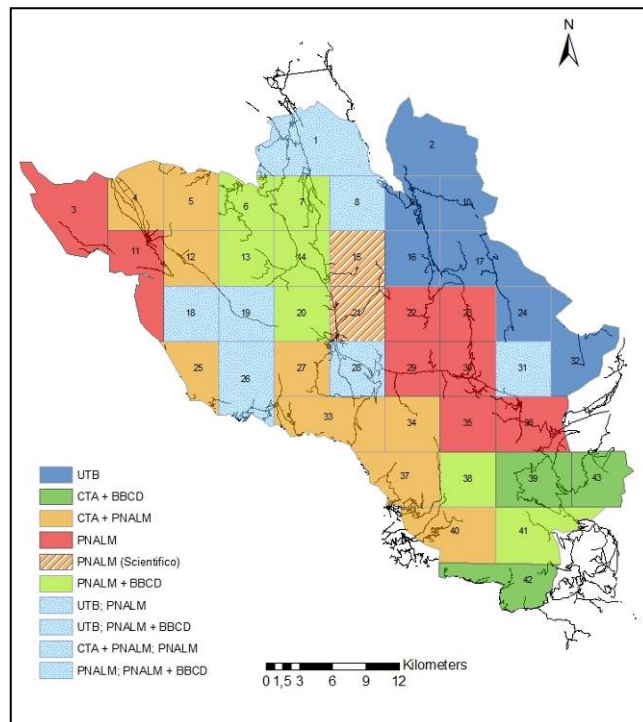


Figure 2. – Spatial partitioning of the 43 hair-snagging grids used for the survey of the Apennine brown bear population (PNALM, 26 May – 26 July 2014). Eleven field teams were simultaneously active (1-12 cells per session), with each team of mixed affiliation (CTA: Forest Service, Coordinamento Territoriale per l’Ambiente; BBCD: Dept. Biology and Biotechnologies University of Rome; PNALM: National Park of Abruzzo Lazio and Molise; UTB: Forest Service, Ufficio Territoriale per la Biodiversità of Castel di Sangro).

Hair-snagging extended for 8 weeks, from May 26 through July 26 2014. Eleven field teams, of 2-5 operators each, worked simultaneously during the hair-snap survey, and they included mixed personnel from the University of Rome, the PNALM authority, and the Forest Service (UTB and CTA) (Fig. 2, Tab. 2). Each field team was pre-assigned a given set of sampling grids, whose location and number (1-12 traps per session) were based on logistics, availability of personnel, and knowledge of the area by the operators (Fig. 2). Each team needed on average 67 (± 49 SD) min to build a hair-trap.

Sampling session	Date		Field teams (n)	Hair traps/team
	from	to		
1	May 26	June 11	10	2 - 8
2	June 6	June 22	11	2 - 9
3	June 17	July 3	11	2 - 10
4	June 28	July 15	11	1-12
5	July 9	July 26	11	2-10

Table 2. – Chronology of the 5 hair-snagging sampling sessions and number of field teams involved to noninvasively survey the Apennine brown bear population in the PNALM ecosystem in 2014.

Similarly to 2011, we used as a lure a 50:50 mixture of cattle blood and rancid fish, pouring about 5 L of it over wood debris piled in the center of the hair traps. The lure was prepared 7 months before (November 2014) and stored within barrels housed in a greenhouse far from the survey area (Fig. 3). To account for the waning in capture probability we observed in 2011, in 2014 we also used secondary lures from the second sampling session onward (Kendall et al. 2008); these were differentiated in subsequent sessions, and included anise extract (session 2), apple (session 3), raspberry (session 4), and a predator long-distance call (K9 Triple Take, treated with trout oil; session 5) (Forsythlure, Alix, Alberta, Canada). Secondary lures were used in addition to the primary lure, hanging on a tree inside each trap one plastic bottle at 2-3 m height containing sheep wool soaked with the lure (Fig. 4).



Figure 3. – The lure for hair-snagging (50:50 mixture of cattle blood and fish oil) was decomposed for 7 months inside barrels housed in a small greenhouse located far from the surveyed area.



Figure 4. – A secondary lure was used from the second hair-snagging session onward to enhance attractiveness of hair traps to bears. Secondary lures (extracts of anise, apple, raspberries, and predator attractant) were soaked in a tuft of sheep wool inserted into small plastic bottles hanging at 2-3 m from a tree along the perimeter of the hair trap.

In contrast to similar hair-snagging surveys (e.g., Kendall et al. 2009), in our bear population cubs are apparently inaccessible to hair-snagging during spring and summer using traditional 50 m-high barbed wire traps (Gervasi et al. 2012). Along with the overall lower capture probability of cubs (Kendall et al. 2009), this is possibly due to their smaller size compared to other brown bear populations, their particularly restricted movements, and the elusive behavior of their mothers, including their lower attraction to lured traps. As a result, no cubs were detected using hair-snagging in previous surveys (Gervasi et al. 2012, Ciucci et al. 2013), although cubs were indeed confirmed in the population in the same years. Following the same rationale we adopted in 2011, we addressed the inaccessibility of cubs to hair-snagging using a double-strand of barbed wire in hair traps located at buckthorn patches only (see below).

2.2.2 Rub-tree sampling

Sampling at rub-trees has been proven to be an efficient way to obtain noninvasive samples from brown bear populations (Kendall et al. 2008, 2009, Stetz et al. 2010, Sawaya et al. 2012), and we confirmed such findings in the 2011 survey (Ciucci et al. 2013). However, due to the highly heterogeneous distribution of rub-trees sampled in 2011, we searched for more rub-trees actively used by bears across the entire surveyed area to be used in the 2014 survey. This searching was conducted by personnel from BBCD and the PNALM, and extended up to 17 August 2014. A total of 44 new rub-trees used by bears was found, contributing to the 191 rub-trees cumulatively inventoried since 2010. At the same time, due to the high sampling redundancy we experienced with rub-trees in the 2011 survey (Ciucci et al. 2013), in 2014 we also applied subsampling procedures to use this sampling strategy in a more cost-effective way; in particular: (a) rub-trees surveyed in 2011 have been subsampled according to their spatial distribution and yield (i.e., number of collected hair samples) in the 2011 survey (Tab. 3), and (b) samples collected per rub-tree and sampling occasion have been subsampled, as in the 2011 survey the largest majority of multiple rub-tree samples belonged to the same bear; Ciucci et al. 2013), with only one sample being sent to WGI for genetic analyses and the other stored as back-ups. Out of the 191 rub trees inventoried in 2014, we installed and visited hair traps in 102 of them, 61 of which were rub trees already used in the 2011 survey and the remaining 42 were used for the first time in the 2014 survey (Fig. 5).

Criteria	Variables	Description
Detection history	Number of genotypes sampled in 2011	Rub tree included if no. genotypes \geq 4
Use by female bears	Genotypes of female bears detected in 2011	Rub tree included if female bears were detected
Rub frequency	Number of successful sampling occasions in 2011	Rub trees more frequently used by bears are preferentially selected
Uniquely detected bears	Number of uniquely detected bears	Rub trees where bears have been uniquely detected are selected preferentially
Sampling area	Number of other rub trees in the general area	Priority is given to rub trees in areas with a low number of additional rub trees
Distribution with reference to the HS sampling grid	Number of rub trees per grid cell	Rub trees within grid cells with no other rub trees are given priority

Table 3. – Criteria used to subsample inventoried rub trees for the 2014 noninvasive survey of the Apennine brown bear population in the PNALM ecosystem (May – September 2014). With respect to the 2011 survey, even though more rub trees have been inventoried in 2014, subsampling was deemed necessary to reduce the sampling redundancy we previously reported, and hence to enhance efficiency of this sampling method (Ciucci et al. 2013).

We attached 4-6 short (30-40 cm each) strands of barbed wire in a zig-zag pattern to the rubbing surface of each rub tree, at about 30 – 170 cm from the tree base (Kendall et al. 2008). As rubbing is a natural behavior, we did not use any attractant to lure bears in. Similarly to the other hair traps, we collected hair samples only from the barbs and passed a flame to avoid contamination between successive sessions. At each sampling occasion, we often found more than one hair sample on the same rub, possibly left by the same bear in a rubbing event. As outlined above, in these cases we subsampled the one sample with more guard hairs and bulbs, even though we often collected up to 2-3 additional samples farthest apart from each other to be used as replicated samples in case the other would not yield a reliable genotype.

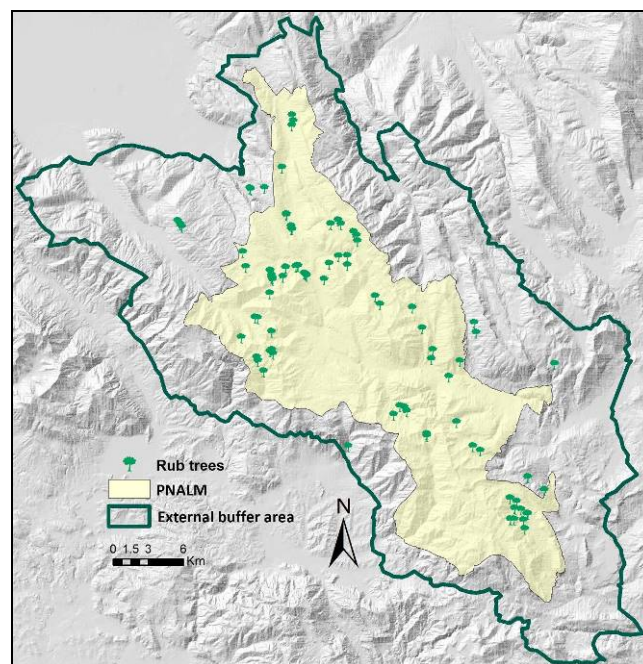


Figure 5. – Distribution of the 102 rub trees used as a secondary sampling method to noninvasively survey the Apennine brown bear population in the PNALM ecosystem (21 May – 30 September 2014).

Similarly to other noninvasive genetic surveys that adopted rub-tree sampling (i.e., Kendall et al. 2009), we started rub-tree sampling in late May and extended sampling through September. Once activated by installing the barbed wire, we visited rub trees every 12-15 days. A subsample of 9 rub trees were additionally monitored with IR camera-traps to better assess use of rub trees by bears and sampling success. We de-installed hair traps at rub trees by the end of the survey.

2.2.3 Opportunistic sampling at buckthorn patches

An effective means to sample our bear population is to opportunistically locate hair-traps at buckthorn patches, where bear congregate in late summer to feed on ripening *Rhamnus* fruit (Gervasi et al. 2008, Ciucci et al. 2013). In addition, as it was done in 2011, by using hair traps at buckthorn sites we intended to include cubs in the final population estimate, as cubs are otherwise invisible to single-stranded hair traps (see §2.2).

Because we had to reduce potential disturbance at buckthorn sites, and also due to logistic constraints, we sampled only a portion of all available buckthorn areas in the PNALM (i.e., 36 in which we detected use by bears during 2004-2008; Ciucci et al. 2013). However, with respect to the 2011 survey, we increased by 2 the number of buckthorn areas to be sampled, while another which yielded no samples in 2011 was substituted with another one, for a total of 9 buckthorn areas sampled in 2014. The three additional buckthorn areas selected for the 2014 survey were chosen based on the number of samples and bear genotypes detected during previous noninvasive surveys (Gervasi et al. 2008, Ciucci et al. 2013).

To avoid disturbance to bears feeding on buckthorn, we installed traps between 1 – 10 August before the ripening of buckthorn berries. In each selected buckthorn area we constructed from 1 to 3 long peripheral hair-traps, each encircling cohesive aggregations of buckthorn patches, for a total of 25 hair traps for all sampled buckthorn areas. The perimeter of individual hair traps at buckthorn patches averaged 27 (± 4 SD) m, ranging 27 – 41 m, excluding one trap of 180 m. It took about 270 (± 35 SD) min for a field crew of 4-9 operators to build traps in each buckthorn site. To increase capture probability of cubs (see § 2.2.1), we used a double strand of barbed wire, at 30 and 50 cm above the ground, and placed at 1.5 m from the nearest buckthorn shrub. As most of these patches occur above timberline, we anchored the barbed wire to steel pegs dug into the ground, using from 6-65 pegs for each trap. Similarly to rub-tree sampling, as feeding on buckthorn patches in a natural behavior for bears, we did not use any attractant to lure bears at hair traps. Due to the remote location and inaccessibility of some of the buckthorn hair traps, the Forest Service made a helicopter available to carry the material.

In 2014, the peak of the *Rhamnus* ripening period was delayed by about 10 days with respect to 2011, and ranged 28 August – 29 September, even though with some variation due to latitude in the surveyed area. We accordingly monitored the status of buckthorn berries, as well as the frequency of bear presence at buckthorn sites, to coincide start of the sampling with the peak of the ripening period. In 2014, all hair traps at buckthorn patches have been activated between 27 and 28 August. We started the first session of sampling by visiting each hair trap and passing a flame under the barb to remove any previously entangled hair tuft. We then checked traps at intervals of 8-10 days, passing a flame under the barbs to avoid contamination between sessions. All hair traps were removed by the time of the last sampling occasion.

2.2.4 Incidental sampling

Hair samples have also been collected by experienced park wardens and forest service personnel during their patrolling activities, including verification of alleged damages by bears. This sampling technique has been previously shown to increase sample size considerably (Gervasi et al. 2008, De

Barba et al. 2010, Ciucci et al. 2013). We extended sampling through this technique across the whole survey period (26 May – 30 September).

All hair-traps used in 2014 have been dismantled by the end of the survey, and all the material used for sampling rub-trees and buckthorn patches has been accordingly removed from the field.

2.3 Genetic analyses

Genetic analyses were conducted at Wildlife Genetic International (WGI) using quality assurance protocols (Paetkau 2003) that have been shown to ensure accurate individual identification (Kendall et al. 2008, 2009). As part of the 2011 survey (Paetkau 2012, Ciucci et al. 2013) we: (a) selected 13 microsatellite markers adequate for individual identification for this bear population, (b) provided empirical evidence of the ideal set of markers to be used in subsequent noninvasive genetic surveys, and (c) provided calibration factors between WGI and the previous Italian lab (ISPRA) as to allow comparison of recent vs. previous genotypes detected in the bear population. Samples collected in the 2014 survey were therefore analysed according to previous guidelines. According to marker selection carried out in 2011, WGI recommend that future analyses of individual identity involving the Apennine bear population use 12 markers, including gender and 11 microsatellites (i.e., all 13 microsatellites used in 2011 except G10X and MSUT-2). In addition, marker *G10P* would also be used to better assess suspicious mismatch cases when comparing sampled between the ISPRA and WGI labs.

2.3.1 DNA extraction

Hair samples were excluded from analysis if they contained no guard hair roots, and <5 underfur. For the samples which were analysed, we aimed to use 10 guard hair roots where available or up to 30 whole underfur, if guard hair roots were lacking. When underfurs were used, the number recorded was an estimate because entire clumps of whole underfur were used rather than clipping individual roots. Hairs were washed in warm water before being placed in the extraction solution. DNA was extracted processing the clippings with QIAGEN DNeasy Blood and Tissue Kits according to the instructions for tissue (for details <http://www.qiagen.com/>).

2.3.2 Marker selection

According to previous marker selection results (Paetkau 2012, Ciucci et al. 2013; see above), we used 11 microsatellite markers plus gender to identify individual bears (Tab. 4). Given the low expected genetic variability of this isolated bear population, these markers are thought to efficiently provide reliable individual identification while allowing comparability with previously scored genotypes by the ISPRA lab. In order to allow this comparison, 9 of the 11 microsatellites used are in common with ISPRA, plus *G10P* that we used at a later stage of the analysis to better discriminate between controversial cases (e.g., 1 MM- and 2 MM-pairs) when comparing genotypes detected in 2014 with the previous ones scored by ISPRA prior to 2011.

WGI uses a scoring convention wherein the database treats 2-digit allele scores as missing data when assigning individual identity. To accommodate this convention, WGI added 100 bp to the allele scores for any marker that has alleles shorter than 100 bp (explaining why 3 markers have scores that differ by roughly 100 bp between labs). For marker MU05, not used before by WGI, scoring was calibrated to match ISPRA existing data. MU11 was treated similarly, but 100 was added to ISPRA allele scores to avoid 2-digit scores for shorter alleles.

Locus	n	H_E	H_O	A	Conversion ^a
CXX20	55	0.62	0.67	3	-. ^b
REN144A06	55	0.61	0.65	3	-. ^b
G1D	55	0.58	0.64	3	+20/+22 ^c
MU51	55	0.57	0.47	3	+92 ^d
G10B	55	0.52	0.47	3	+28
G10C	55	0.50	0.56	3	+102 ^d
MU59	55	0.49	0.53	2	+128
MU05	55	0.47	0.47	2	0
G10L	55	0.44	0.51	2	+9
MU50	55	0.44	0.47	2	+32
MU11	55	0.44	0.38	2	+100 ^d

G10P	24	0.22	0.25	2	-7

<i>11-Locus mean</i>		<i>0.52</i>	<i>0.53</i>	<i>2.6</i>	
<i>(±S.D.)</i>		<i>(±0.07)</i>	<i>(±0.09)</i>	<i>(±0.52)</i>	

^a: to obtain WGI score from ISPRA score

^b: not used by ISPRA

^c: ISPRA ≤ 150 bp, and > 150 bp, respectively

^d: markers with alleles <100 bp actual length are scored 100 bp higher at WGI to accommodate use of 2-digit allele scores for low-confidence (≈failed) results

Table 4. – Measures of variability including the observed number of alleles (A), and expected (H_E) and observed (H_O) heterozygosity of the 11 markers (plus gender) used at WGI for individual multilocus genotyping. Nine of the 11 markers are in common with the ISPRA lab (all except *CXX20* and *REN144A06*). Conversion factor are also provided, representing the amount to add or subtract to the ISPRA allele scores to convert them to WGI scoring (see also Ciucci et al. 2013). *G10P*, also in common with ISPRA, has been used to better discriminate equivocal cases (i.e., 1-MM and 2-MM pairs) when comparing genotypes detected in 2014 with the previous ones scored by ISPRA.

2.3.3 Microsatellite genotyping

Analysis of the hair samples started with a first pass during which all extracted samples were analyzed at 6 of the 12 markers (11 microsatellites plus gender). After first pass we culled samples that had high-confidence scores for ≤ 3 of 6 markers, by using a combination of objective (i.e. peak height) and subjective (i.e. appearance) criteria to classify genotype scores (Paetkau 2003). In WGI experience, no amount of effort will produce complete, accurate genotypes from such samples. The first pass was followed by a clean-up phase in which WGI re-analyzed data points that were weak or difficult to read the first time (i.e. scored with low-confidence, 2-digit alleles), using 5 µl of DNA per reaction instead of the 3 µl used during the first pass. In some cases multiple rounds of reanalysis were used to confirm persistently weak data points. This process (first pass and clean-up) was then repeated at the other 6 markers with the non-culled hair samples, and further samples were eliminated after the clean-up phase of this second round of 6-locus genotyping. Samples left after this final cull had high-confidence scores for all 12 markers.

Multilocus analysis finally addressed error-checking, where we searched for and re-analyzed any pair of genotypes that was similar enough to have conceivably been created by genotyping error (Paetkau 2003). Intensive testing with blind control samples has shown that this protocol effectively prevents the recognition of false individuals through genotyping error (Kendall et al. 2009), although it does not claim to eliminate genotyping errors in cases where only one sample has been analyzed from a given individual. During error-checking, 5 errors were found and corrected, of the sort expected when working with sparse DNA sources like hair follicles. After correcting these errors, the most similar pair of genotypes corresponded to one 1-MM pair and 3 2-MM pairs, and those mismatching data points had been solidly replicated to rule out genotyping error.

The last quality control phase involved interaction between the genetic lab (WGI) and field staff (BBGD), as already done in previous genetic sampling projects on the Apennine brown bear (Gervasi et al. 2012). We performed a series of cross-controls between the information provided by the

genotyping process and that included in field data (dates and locations of sample collection, GPS data from radio-collared bears, position of samples on the trap, etc.). The aim was to further investigate some cases from the 2011 survey (i.e., unique samples, some mismatching) by: (a) comparing locations of samples attributed to previously radio-collared bears with GPS locations from the same bears; (b) plotting the distance and time between samples attributed to the same genotype, to check if any samples had been collected at unexpectedly large distances; (c) checking the consistency of the results at each trap, evaluating dates of collection, the position of samples on the trap, and the number of mismatching loci among genotypes sampled at the same trap.

We also cross-checked any 1 MM-, 2MM- and 3 MM-pairs, or other potentially equivocal results, which emerged during comparison of multilocus genotypes detected in 2014 with those detected in previous surveys, including those previously scored by ISPRA (2000 – 2008). To this aim, we added in this step of the analysis the *G10P* marker to better discriminate between samples that had suspicious MM-pairs between labs. Evaluation of these cases has been based on the number and type of samples (i.e., hairs vs. scats) and markers involved, sampling dates, re-sampling rates, and geographic appraisal of their distribution.

2.4 CR modelling and model selection

We used Huggins closed population models (Huggins 1991) in Program MARK (White and Burnham 1999) to estimate the size of the Apennine brown bear population. As a first step, we combined data from the 4 non-invasive sampling methods described above to construct individual encounter histories. For each sampled bear, we recorded hair-snag captures in sessions 1-5, captures at buckthorn aggregations in sessions 6-9, rub tree samples in sessions 10-19, and incidental genetic samples in session 20. This approach is allowed in a context of closed population capture-recapture models, as the relative order of sessions is irrelevant to parameters estimation, unless any behavioural response is expected in the data (Boulanger et al. 2008). In our case, we assumed our sampling design to be minimally affected by any behavioural response. The issue did not involve incidental samples, as this data source was summarized into a single session, thus a-priori preventing any possible response. As to hair-snag, data traps were moved between successive sessions, providing no reward to sampled bears, whereas both buckthorn and rub tree sampling took advantage of a natural behaviour by bears, without enhancing or stimulating it in any way (Gervasi et al. 2012).

After building the encounter histories, we constructed candidate models for each data source, and combined them into a most parameterized starting model. The variables included in the initial most parameterized model were selected based on a-priori knowledge of bear biology and spatial behaviour, on previous non-invasive applications in North America (Boulanger et al. 2008, Kendall et al. 2008), and on our own previous experience in sampling this bear population (Gervasi et al. 2010, 2012, Ciucci et al. 2013). These variables are summarized in the following sections for each data source.

2.4.1 Hair-snag data

For the hair-snag sampling, we first tested for a temporal variation in capture probability, both through a simple *time effect* (one parameter for each session) and through a *trend effect*, aimed at detecting a linearly increasing or decreasing capture probability during the whole survey. We also compared models with similar and different capture probabilities for the two sexes. We then tested if capture probability was different between bears with and without a hair snagging event during previous years, and as a function of the total number of previous hair-snag detections since 2003. As many of the bears born before 2011 had been sampled at least once through hair snag (5 noninvasive survey attempts have been conducted between 2003 and 2011 in the same area), both

the above variables were expected to be correlated with a bear's age. Because bears detected in 2011 or previous years are expected to be ≥ 4 years old in 2014, most of the bears detected in 2014 with no previous hair-snag history were expected to be younger than 4 years (i.e., born after 2011), although they may also include older bears that went undetected during previous surveys. Based on the hair-snag capture probability estimates of the 2004, 2007, 2008 and 2011 surveys, the probability for a 4 years old male bear to be correctly classified was 83%, whereas the same probability for a female of the same age was 76%. These probabilities further increased for bears older than 4 years (Appendix A). A potential confounding factor in such an assessment was the probability that bears older than four years had immigrated in the study area after 2011. Unfortunately, the potential role of immigration could not be evaluated due to the lack of empirical data on this process in the PNALM, although it is believed to occur at negligible levels for the purpose of our analysis.

2.4.2 Buckthorn data

Similarly to hair-snag data, we tested a simple *time effect* (one parameter for each session) and a *trend effect* also for the buckthorn sampling. As the buckthorn sampling was performed during an overall period of about one month, we expected that the progression of the *season*, affecting the ripening of berries and the extent of use of buckthorn aggregations by bears, could generate different capture probabilities among sessions. As the length of each session was slightly different among sessions and sampling sites, we also assessed if the temporal variation in sampling effort during the 4 buckthorn sessions affected the variation in capture probability. Accordingly, as an estimate of effort, we used the cumulative number of trap nights in each session multiplied by the cumulative length of the barbed wire of all traps by each buckthorn site. Also, as the study area lies on a broad NW-SE gradient, we expected the ripening of buckthorn berries to occur later in the season in the Northern part; we therefore tested an interaction between the time effect and the *latitude* of the central sampling point of each bear. Finally, we included a *sex effect* and an effect of a previous hair-snag sampling (see above) as a crude proxy of age class under the same hypotheses described for the hair-snag sampling. Finally, we also tested if bears living farther from buckthorn aggregations had a reduced capture probability for this data type than bears living close to buckthorn areas. To this aim, we calculated for each bear the geometric centre of all its sampling locations and the distance from the closest buckthorn aggregation. Then we used this individual covariate to model capture probability for this data type.

2.4.3 Rub tree data

Rub tree sampling was modelled according to 10 13-day sessions, from the 1st of June to the end of September. The heterogeneity in sampling effort per session was modelled according to the cumulative number of rub tree sampling nights for each session (Kendall et al. 2008). We also modelled additional temporal variation in capture probability with 2 alternative variables: *i*) a simple *time effect* (one parameter per session); *ii*) a *trend effect*. In addition, because of the uneven distribution of installed rub trees, we expected the spatial variation in capture probability to be markedly affected by variation in sampling effort (i.e., number of installed rub trees in different portions of the study area); to model it, we first calculated the centre of all sampling locations for each bear; we then created a buffer equivalent to the average seasonal home range, for males and females separately (115 and 50 km², respectively; Tosoni 2010), and finally calculated the number of RTs in each individual "home range" weighted (i.e., multiplied) by the actual sampling nights (RT-nights within individual home ranges). We also included a *sex effect* and an effect of a previous hair-snag sampling (see hair snag data), and tested for a possible interaction between these two variables. As described above, the binary variable separating bears with previous hair-snag events from the ones never detected before through this method was expected to be highly correlated to a

bear's age class. As the use of rub trees is known to be especially frequent in adult male bears (Green and Mattson 2003), we expected the interaction effect to be supported by the data.

2.4.4 Incidental sampling data

For the incidental samples we had reduced modelling opportunities, as we did not have direct estimates of sampling effort. We therefore pooled all data in a single session, and tested for a *sex effect*, the effect of previous hair-snag detection (see above), and finally for an effect of the linear distance between the mean detection location through this sampling method and the closest village. This last hypothesis accounted for a higher expected propensity of some bears to visit villages within the PNALM and damaging properties (i.e., crops, beehives, livestock) and being subsequently detected through incidental sampling as this includes samples collected during verification by park wardens of alleged claims by farmers.

We also tested the effect of spatial variables potentially affecting variation in capture probability for all sampling techniques simultaneously. Accordingly, for all data sources we tested if bears living closer to the border of the sampling area had a reduced capture probability, to model for the possible violation of the geographic closure assumption, which would generate a decreasing capture probability in the peripheral part of the study area. To do this, we pooled all captures for each individual and used the distance of the centre of these locations from the closest grid edge (DFE) to assess if some closure violation was supported by the data. We also tested a $\text{Log}(\text{DFE})$ and DFE^2 functions to assess if different shapes of the relationship were more supported by the data. In addition, we tested for a possible difference in sampling efficiency across different portions of the study area: this hypothesis accounts for the likely effect of our ability to identify ideal trap sites on sampling performance differentially for some data sources (especially hair-snag and rub trees), as we would expect that sampling efficiency was higher toward the central part of the study area with respect to the external portions. As the main backbone of the study area (the main Apennine divide) broadly lies on a NW-SE gradient, and because most field and patrolling activities were concentrated in the proximity of the divide, we estimated for each bear the distance of its mean sampling location from the NW-SE backbone of the study area (DFC).

To avoid modelling problems due to collinearity among predictors, we calculated pairwise correlation for all the explanatory variables, and an overall Variance Inflation Factor (VIF) for the whole set of variables included in the analysis. This preliminary exploration of collinearity revealed a high degree of positive correlation between the distance from the closest buckthorn patch (*DFB*) and that from the main mountain ridge at PNALM (*DFC*; Spearman's $r = 0.81$), and between *DFC* and the number of rub trees in a bear's home range (*nrub*; Spearman's $r = 0.79$). Therefore, we did not test correlated variables in the same model.

After generating the most parameterized general model, we fitted reduced models and assessed their relative 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). Starting from the most parameterized model, including all the above described effects and interactions for each data type, we then fitted less parameterized models for the hair-snag part only, while keeping the same structure for the rest of the design, and we identified the most parsimonious parameterization for this data type. Once the most supported variables were identified for the hair-snag part, we kept them constant for the rest of the model selection procedure, and repeated the same model selection approach with each of the remaining parts of the analytical design, thus finally identifying the most parsimonious general model. To account for the degree of uncertainty in model selection, we model averaged parameter estimates from all the fitted models, 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

(White et al. 2002), accounting for the minimum number of bears known to be alive and in the study area, through all the available sampling methods.

3. RESULTS

3.1 Collected samples

Overall, from 26 May to 30 September 2014, and based on all noninvasive genetic sampling techniques, we collected 476 samples. Most (43.5%) of these were obtained by rub tree sampling, followed by HS (24.8%), buckthorn (17.0%) and incidental (14.7%) sampling. About 99% of collected samples (n=466) were sent to the genetic lab for multilocus genotyping, varying from 92.6% of buckthorn samples to 100% of systematic samples. We also sent to WGI an additional hair sample, collected from a cub found dead on August 2014, and three tissue samples collected from bears found dead in past years and that still needed to be individually identified. Another bear was illegally shot immediately outside the eastern edge of the sampling grid on 12 September 2014, and its genotype, scored by ISPRA, was kindly made available by M. Fabrizio (Genzana Nat. Reserve) to be compared with those we detected in the previous weeks in our study area.

3.1.1 Systematic hair-snagging

We hair-snagged bear samples in 28 (65.1%) out of 43 sampling cells, reflecting bear distribution across the entire study area (Fig. 6). Most (75.9%) successful grid cells provided bear samples in only 1 session, whereas 17.2% provided samples in 2 sessions, and an additional 6.9% in 3 sessions. From 4 to 14 traps provided samples in each sampling session, for a total of 37 successful hair traps out of 215 during all 5 sessions (17.2%). We cumulatively collected 118 bear samples during the 5 sampling sessions, ranging 13 – 52 samples per session, with an average (\pm SD) of 0.55 (\pm 0.4) samples per trap (Table 5). All 118 collected bear samples were delivered for genetic analyses.

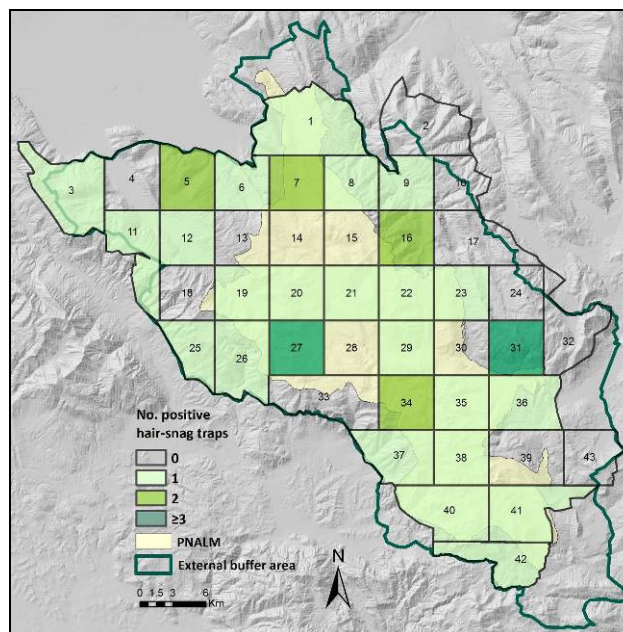


Figure 6. – Hair-snagging sampling grid adopted for the survey of the Apennine bear population (26 May – 26 July 2014), and grid cell distribution based on the number of successful traps per grid. Sampled area encompass the PNALM and portions of its external buffer area and has been designed along topographic, habitat and anthropogenic features to ensure population closure.

Sampling session	Date ^a	Successful grid cells ^b	Successful traps ^b	Bear samples	Bear samples/trap	
					mean	range
1	26 May – 6 June	14 (32.6%)	14 (32.6%)	52	1.21	1 – 8
2	8 – 19 June	4 (9.3%)	4 (9.3%)	13	0.30	1 – 4
3	20 June – 1 July	5 (11.6%)	5 (11.6%)	13	0.30	1 – 7
4	2 July – 14 July	9 (20.9%)	9 (20.9%)	25	0.58	1 – 10
5	15 – 26 July	5 (11.6%)	5 (11.6%)	15	0.35	2 – 4
<i>Total</i>		<i>29 (67.4%)</i>	<i>37 (16.7%)</i>	<i>118</i>	<i>0.55 (±0.4)</i>	<i>1 – 10</i>

^a: because the date of deactivation of the trap could have been anticipated or postponed by 1-2 days, the average length of each sampling session (12 days) might have varied ±2 days

^b: in parenthesis percentage of successful grids/traps per sampling session

Table 5. – Results of hair-snag sampling by sampling session (Apennine bear population survey in the PNALM, 26 May – 26 July 2014).

3.1.2 Rub-tree sampling

In 2014 we installed with hair traps a total of 102 rub trees up to 17 August, even though 95% of them was installed by June 15. The overall sampling period (26 May – 2 October 2014) ranged from 38 to 131 days per rub tree ($\bar{x} \pm SD = 117 \pm 14$ days/rub tree), during which we visited installed rub trees at an average frequency of 11 (± 2 SD) visits per rub tree. Nine activated rub trees were also discontinuously monitored by means of camera-trapping (July – October 2014), all of which provided a total of 8 video clips of bears rubbing or investigating rub trees (see §§3.2.5 and 3.3.4).

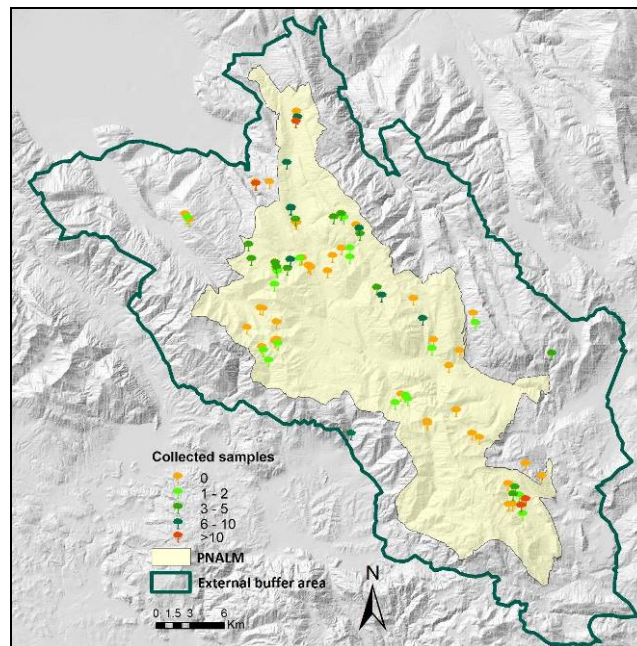


Figure 7. – Distribution of rub trees that have been armed with hair traps (n=102) to survey the Apennine brown bear population in the PNALM ecosystem (21 May – 30 September 2014).

Overall, we collected 207 bear samples by rub tree sampling, including 115 main samples and 92 back-ups (i.e., samples believed to belong to the same bear already sampled in the same sampling occasion). Forty-eight (47.1%) of the armed rub trees provided bear samples on at least one sampling session (Fig. 7), for an average of 4.3 (± 3.7) bear samples/successful rub tree, ranging 1-16 samples/rub tree. For modelling purposes, we arbitrarily divided the sampling period in 10 13-day sampling sessions (Table 6). From 7 to 17% installed rub trees proved successful, providing an

average of 0.24 (± 0.77 SD) bear samples/activated rub tree per session (Table 6). As the 207 collected bear samples included 3 inadequate samples (few hairs and/or no bulb), only 204 were sent for genetic analysis, comprising the 93 back-up samples.

Sampling session	Date ^a	Installed rub trees (no.)	Rub tree effort ^b	Rub trees with bear hair ^c	Bear samples	No. bear samples/rub tree ^d
1	26 May – 7 June	20	220	2 (10.0%)	5	0.23 (± 0.73)
2	8 – 20 June	94	1429	9 (9.6%)	20	0.21 (± 0.76)
3	21 June – 3 July	94	1251	14 (14.9%)	30	0.31 (± 0.89)
4	4 – 16 July	95	1267	13 (13.7%)	32	0.34 (± 0.94)
5	17 – 29 July	100	1390	17 (17.0%)	37	0.37 (± 0.99)
6	30 July – 11 Aug	94	1097	8 (8.5%)	15	0.16 (± 0.59)
7	12 Aug – 24 Aug	97	1231	14 (14.4%)	25	0.26 (± 0.71)
8	25 Aug – 6 Sept	96	1248	10 (10.4%)	17	0.18 (± 0.60)
9	7 – 19 Sept	82	1129	6 (7.3%)	15	0.18 (± 0.78)
10	20 Sept – 2 Oct	97	1562	7 (7.2%)	11	0.11 (± 0.43)
<i>Total</i>		<i>101</i>	<i>11824</i>	<i>48 (47.5%)</i>	<i>207</i>	<i>0.24 (± 0.77)</i>

^a: for modeling purposes, sessions were arbitrarily defined as 13-day intervals

^b: cumulative number of installed rub trees multiplied by the number of days each has been surveyed within the session

^c: in parenthesis percentage of successful rub trees per sampling session

^d: mean \pm SD per activated rub tree (i.e., including rub tree from which no samples were obtained)

Table 6. – Results of rub tree sampling by sampling session (Apennine bear population survey in the PNALM ecosystem, 26 May – 2 October 2014).

3.1.3 Opportunistic sampling at buckthorn patches

We cumulatively installed 25 hair traps in 9 buckthorn sites (Fig. 8, Tab. 7). All traps have been activated between 27 and 28 August, at the peak of the *Rhamnus* ripening period. On average, we visited traps every 8 (± 1.5 SD) days, for a total of 4-5 visit at each buckthorn site. Total sampling period by buckthorn site ranged from 30 to 33 days, for an average of 32 (± 1) days per site (Table 7).

Buckthorn site ^a	Code	No. traps	Total trap length (m)	Sampling period		No. visits		Bear samples
				from – to	days	total	successful	
Monte Marrone	RAM_001	1	180	28 Aug – 28 Sept	31	4	3	11
Valle Orsara	RAM_003	3	65	28 Aug – 27 Sept	30	4	0	0
Pozzo Neve	RAM_004	3	80	28 Aug – 29 Sept	32	5	4	36
Argatone	RAM_005	3	80	28 Aug – 29 Sept	32	5	1	1
Valle Celano	RAM_006	3	90	28 Aug – 30 Sept	33	4	2	11
Ortella	RAM_007	3	100	28 Aug – 29 Sept	32	4	2	7
Guadarola	RAM_008	3	65	27 Aug – 28 Sept	32	4	0	0
Rocca Altiera	RAM_009	3	80	28 Aug – 29 Sept	32	4	0	0
Capriola	RAM_010	3	80	28 Aug – 30 Sept	33	4	3	15
<i>Total or mean(\pmSD)</i>		<i>25</i>	<i>820</i>		<i>32</i> <i>(± 1)</i>	<i>4.2</i> <i>(± 0.4)</i>	<i>1.7</i> <i>(± 1.5)</i>	<i>9.0</i> <i>(± 11.6)</i>

^a: see Figure 8 for location of buckthorn sites

Table 7. – Results of noninvasive sampling at the 9 buckthorn sites as a complementary sampling method to survey the Apennine bear population in the PNALM ecosystem in 2014. Each site was activated with two strands of barbed wire encircling most productive buckthorn patches, and 1-3 traps of different perimeter length were activated per site.

Overall, 66.6% (n=6) of the installed buckthorn sites proved successful, each providing on average of 9.0 (± 12) bear samples, ranging from none to 36 (Table 7), for a total of 81 collected bear samples. During each session, successful buckthorn sites provided 1 – 19 bear samples per site at a mean rate of 5.6 (± 2.4) samples/site, although with high variability from session to session (Table 8). Out of the 81 collected samples, only 75 were considered feasible for genetic analyses.

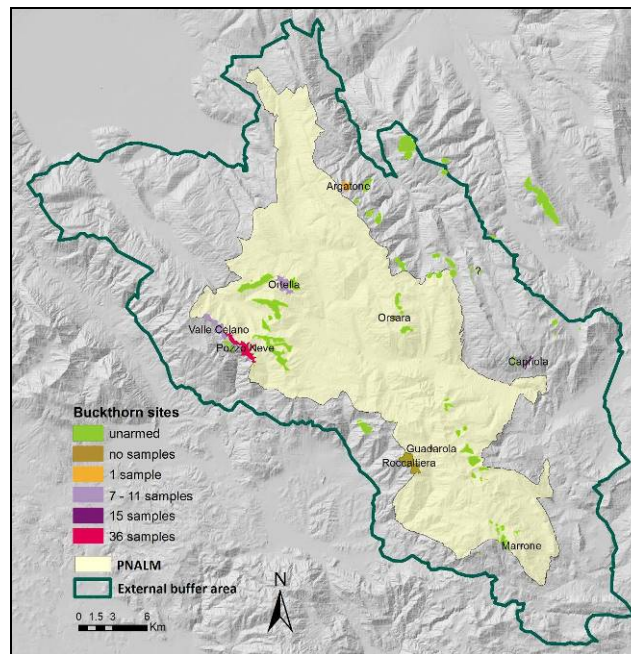


Figure 8. – Distribution of relevant buckthorn sites in the PNALM (n=36), 9 of which were instrumented with hair traps to survey the Apennine bear population (27 Aug – 30 Sept 2014). Armed buckthorn sites are ranked according to their sampling success (bear samples collected across the whole sampling period) (cf. Table 7).

Sampling session	Date from - to	Successful sites ^a	Sampling effort ^b	Bear samples	Bear samples/site	
					mean	range
1	27 Aug – 4 Sept	1 (11%)	405	8	0.9 (± 2.5)	0 – 8
2	5 – 13 Sept	4 (44%)	8124	37	4.1 (± 6.1)	0 – 19
3	14 – 22 Sept	6 (67%)	9066	26	3.9 (± 3.1)	0 – 9
4	23 – 30 Sept	3 (33%)	6537	9	1.0 (± 1.6)	0 – 5
<i>Total</i>		6 (67%)		80	2.2 (± 3.9)	0 – 19

^a: in parenthesis percentage of successful buckthorn sites per sampling session

^b: total number of trap-nights per session multiplied by length of barbed wire installed

Table 8. – Results of opportunistic sampling at buckthorn sites by sampling session (Apennine bear population survey in the PNALM ecosystem, 27 August – 30 September 2014).

3.1.4 Incidental sampling

From 11 June through 27 September 2014, 70 bear samples were collected incidentally to patrolling and management activities, including verification of alleged damages to livestock and crops by bears, as well as during other field activities (Fig. 9). Samples collected incidentally were broadly distributed across the survey area, although 9 of them fell outside the hair-snagging grid, with distances beyond the closest grid edge ranging 0.2 – 6.6 km (Fig. 10). Sixty-nine (57 main samples plus 12 back-ups) of the incidentally collected samples were delivered for genetic analyses.

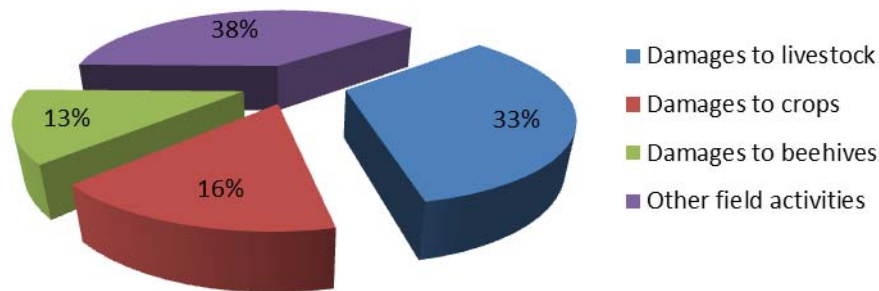


Figure 9. – Distribution of 70 hair samples collected incidentally during patrolling or management activities by park wardens or during other field activities (PNALM, June – September 2011).

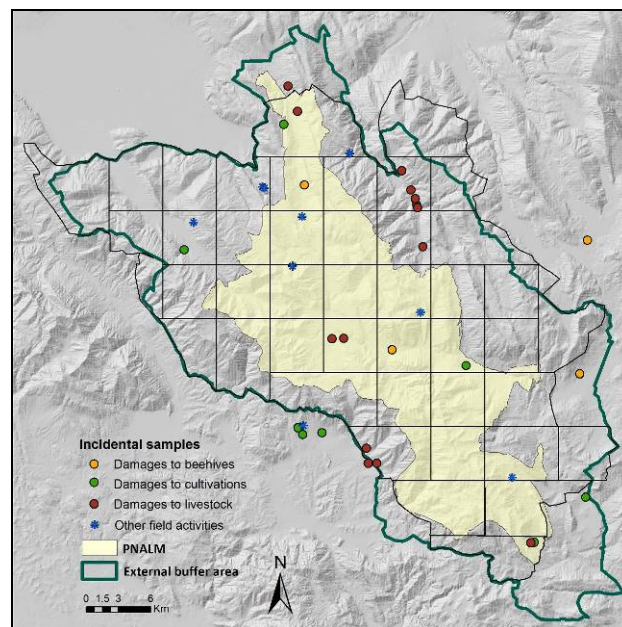


Figure 10. – Distribution of 70 bear hair samples incidentally collected during field work and patrolling activities, including verification of alleged damage claims to cultivation, livestock and beehives in the PNALM ecosystem (11 June – 27 September 2014). More samples were collected at the same location. Not all samples yielded DNA results (see § 3.3.1) and 9 were collected 0.2 – 6.6 km outside the sampling grid.

3.1.5 Tissue and hair samples from dead bears

In addition to noninvasively collected samples, in 2014 we also sent to WGI 4 samples extracted from bears found dead (Table 9). These comprised three adult bears found dead from 2002 to 2012, and whose genotype was never scored until now, and one cub that was found dead from unknown causes on 28 August 2014. With reference to the latter, we were interested in knowing if this bear had been sampled in 2014 before it died, as to eventually remove it from the CR encounter history; for the other three bears we were interested in comparing their genotypes with those of all bears noninvasively sampled since 2000.

Bear code	Date retrieval	Sample type
Orsnec0302	October 2, 2002	frozen tissue
Orsnec0210	June 12, 2010	frozen tissue
Orsnec0112	May 30, 2012	frozen tissue
Orsnec0114	August 28, 2014	hair tuft

Table 9. – List of bears found dead in the PNALM ecosystem whose tissue or hair samples have been sent to WGI for multilocus genotyping.

3.2 Genetic analyses

3.2.1 Success rate and culled samples

In total, 466 alleged bear hair-samples collected by all four sampling techniques have been considered for genetic analyses, including 105 replicated samples (92 from rub tree sampling, 12 from incidental sampling, and 1 from buckthorn sampling; Table 10). Out of these, 88 samples have not been used since they were replicates of main samples that were analyzed successfully, and 32 were discarded as they contained no guard hair roots and <5 underfur; many of these were broken guard hair shafts lacking roots, the majority of which from buckthorn samples (n=15).

	Total	Sampling method			
		HS	RT	OPP	INC
Collected	476	118	207	81	70
<i>to lab</i>	466	118	204 ^a	75 ^b	69 ^c
<i>replicates not analyzed</i>	88	-	77	1	10
<i>inadequate</i>	32	9	7	15	1
Analyzed	346	109	120	59	58
<i>culled</i>	70	17	32	7	14
<i>successful</i>	276	92	88	52	44
<i>% successful</i>	79.8%	84.4%	73.3%	88.1%	75.9%

^a: including 92 alleged back-up samples (i.e. replicated samples from the same rub tree sampling event)

^b: including 1 alleged back-up sample

^c: including 12 alleged back-ups

Table 10. – Descriptive statistics of 476 bear hair samples noninvasively collected to estimate population size of the Apennine brown bear in the PNALM ecosystem (June – September 2014). We used four sampling methods: (HS) systematic hair snagging; (RT) rub-tree sampling; (OPP) opportunistic sampling at buckthorn patches; (INC) incidental sampling.

Of the remaining samples which were analyzed (n=346), 70 were culled whereas 276 samples, including 17 replicates of failed main samples, had high-confidence scores for all 12 markers (Fig. 11). Overall success rate was 79.8%, ranging from 73.3% by rub tree sampling to 88.1% by buckthorn sampling, with incidental sampling and hair-snagging at intermediate values (Table 10).

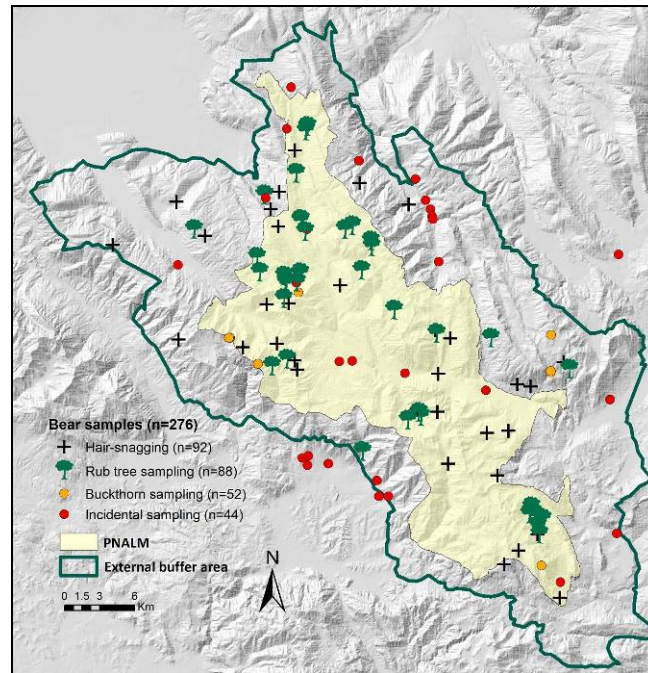


Figure 11. – Distribution of the 276 successfully genotyped bear samples, based on four sampling strategies to survey the Apennine bear population in the PNALM ecosystem (June – September 2014). More than one samples were collected at the same location.

Although proportion of culled samples tended to be disproportionately higher for rub tree and incidental sampling (Fig. 12), these differences were not significant and we therefore obtained similar proportions of collected vs. successfully analyzed samples by sampling method ($5.2 \leq G_{adj} \leq 6.5$, d.f.=3, $0.088 \leq p \leq 0.158$). Excluding unused back-up samples, rub tree sampling had the highest share of inadequate and failed samples, cumulatively accounting for 30.7% of samples collected at rub trees, whereas hair-snagging had the lowest (22% of collected samples; Table 10).

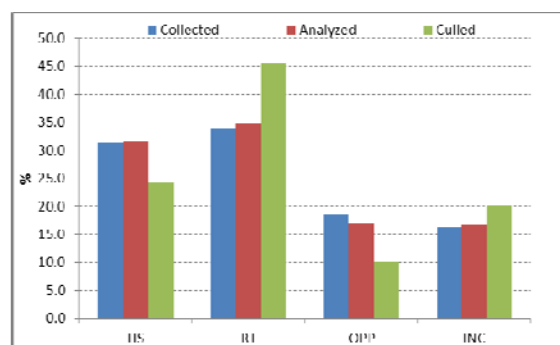


Figure 12. – Distribution, by sampling method, of bear hair samples which have been collected and successively used (successful) or culled for genetic analysis (PNALM, June – September 2014).

On average, there were 5.9 guard hairs per analyzed sample (counting underfur as 0.2 guard hairs). Buckthorn samples were sparser than average, with an average of 4.0 guard hairs per analyzed sample, even though they had the best success during genotyping (88%). By contrast, rub tree samples had a mean of 5.8 guard hairs per extract but only 73% were genotyped successfully.

In terms of post-quality control, all the samples attributed to previously collared bears were consistent with their estimated home-ranges during the previous years, and all noninvasive samples attributed to the same individual were spatially distributed within expected distances and at

reasonable patterns, revealing no suspicious cases. We detected only one potential exception, a 2014 single, buckthorn sample matching bear RAM 024, already sampled in 2011, but detected at 27 km north of its 2011 sampling location: this sample has been re-analyzed, including additional markers Msut-2 and G10X, confirming its matches with bear RAM 024 and revealing a possible case of short-distance range displacement.

3.2.2 Marker power for individual identification

WGI preferred approach to estimating the probability of sampling more than 1 individual with a given multilocus genotype is to extrapolate from an observed mismatch distribution (Paetkau 2003). This empirical approach provides more meaningful insight than calculated match probabilities, because the distribution of degrees of relatedness among the sampled individuals — an unknown parameter that has a profound effect on calculated match probabilities — is implicit in the mismatch distribution. By merging all bear genotypes scored by WGI and detected both in the 2011 and the 2014 surveys, the current 12-locus dataset of 78 individuals contains just 1 pair of genotypes that match at 11 of 12 markers (a '1MM-pair'), and 3 2MM-pairs (Fig. 13). The 1MM- and 2MM-pairs were replicated during error-checking, to demonstrate that these similar pairs did not arise through genotyping error. Even with the high consanguinity expected in a population of this size, and despite the low variability, matches at all 12 markers are less likely than 1MM-pairs. This suggests that the current marker system is likely to uniquely identify each surveyed individual.

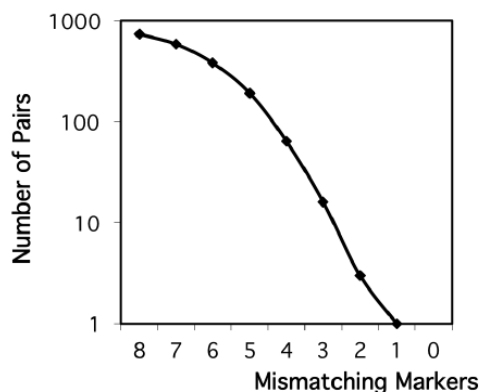


Figure 13. – Mismatch distributions for the 78 unique 12-locus genotypes in the current WGI database of the Apennine brown bear. Extrapolation suggests that the risk of false matches within this dataset is still well controlled, although loss of variation over time could change this. The 1MM- and 2MM-pairs were replicated during error-checking, to demonstrate that these similar pairs did not arise through genotyping error.

3.2.3 Bear genotypes detected in 2014

The 276 hair samples which provided successful genotypes were assigned to 44 bears¹ (Appendix B). Of these, 20 were males and 24 were females, with a sex ratio of 1:1.20 males to females. Twenty-nine of the detected bears were recaptures from previous surveys, whereas 15 were bears never detected before. Bears were sampled from 1-29 samples each (Fig. 14); ten bears were sampled only once, including 5 previously sampled bears and 5 bears that were never sampled before (cf. Fig. 14). We detected 23 bears through hair-snagging, 22 by rub tree sampling, 13 by buckthorn sampling, and 19 by incidental sampling (Table 11). One bear was sampled by all four sampling methods, 8 by any combination of three methods, 14 by any combination of 2 methods, and 21 bears by one sampling method only; of these, 4 were sampled by HS only, 5 by RT only, 6 by RAM only, and 6 by ACC only (Table 11).

¹ However, only 43 bears were included in the encounter history used for the estimation of population size; one bear was removed from the analysis as it was detected at more than 6 km outside the sampling grid (cf Fig. 10 and 11).

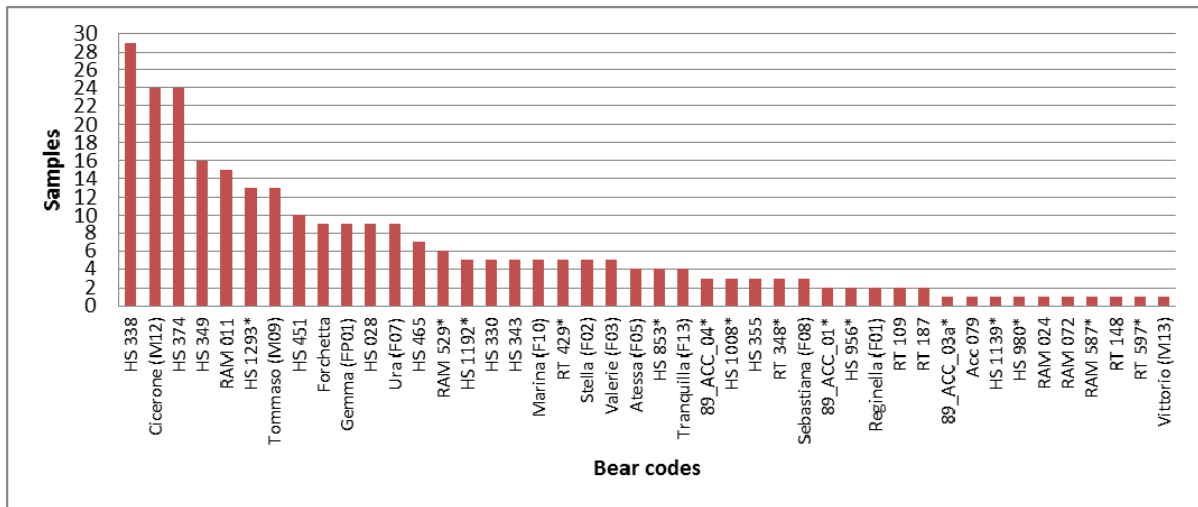


Figure 14. – Sampling frequency of 44 bears noninvasively detected from June – September 2014 in the PNALM ecosystem. Fifteen bears (marked with *) were never detected in previous surveys.

	Total	Sampling method			
		HS	RT	OPP	INC
Genotypes	44	23	22	13	19
Unknown genotypes ^a	15	7	5	3	3
Unknown/all genotypes (%)	34.1%	30.4%	22.7%	23.1%	15.8%
Uniquely detected genotypes ^b	21	4	5	6	6
Genotypes sampled only once ^c	10	2	3	2	3
Genotypes/analyzed sample	0.13	0.21	0.18	0.22	0.33
Uniquely detected genotypes/ analyzed sample	0.06	0.04	0.04	0.10	0.10

^a: number of genotypes unknown from previous non-invasive surveys (2000 – 2008) and live-trapping projects (2006 – 2010)

^b: number of genotypes uniquely sampled by a given sampling method

^c: 5 of which already sampled in previous surveys

Table 11. – Descriptive statistics of 476 bear hair samples noninvasively collected to estimate population size of the Apennine brown bear in the PNALM ecosystem (June – September 2014). We used four sampling methods: (HS) systematic hair snagging; (RT) rub-tree sampling; (OPP) opportunistic sampling at buckthorn patches; (INC) incidental sampling.

We detected the highest number of genotypes through hair-snagging, which also accounted for the highest number of previously unknown genotypes (Table 11). This is expected based on the intensive and widely distributed sampling effort throughout the study area that theoretically should account for a non zero probability of detected for all bears. In terms of detected genotypes, efficiency of sampling was however comparable among sampling methods, as they required from 3 (incidental sampling) to 5 (all other sampling methods) samples to detect a genotype. The complementary sampling methods, with respect to hair-snagging, contributed proportionally more in terms of uniquely detected bears: whereas we needed 24 hair-snagged samples to uniquely detect a bear, buckthorn and incidental sampling were more efficient in this regard as they contributed a unique bear every 10 samples each (Table 11).

As in 2011, also in 2014 we confirmed that complementary sampling methods accounted for cubs, while we have reasons to believe that cubs remain inaccessible to hair-snagging alone. In particular, out of 3 samples incidentally collected at a site previously visited by a known family group including known female F05 and her 3 cubs, 2 were successfully scored revealing F05 and another genotype. The latter was consistent with one of F05's offsprings as it was never sampled in previous surveys (2000 – 2011), it shared at least one allele with F05 at all 11 loci (Table 12)², and it was sampled together with F05 in the same sampling occasion. Re-analysis of the third sample, even from single hairs, did not result in high-confidence scores for all 11 markers and was therefore discarded.

Genotype	G10B	G10C	G1D	G10L	MU59	REN144A06	CXX20	MU50	MU51	MU05	MU11	Sex
F05	140.156	203.207	186.186	163.163	229.229	109.109	137.139	132.132	206.206	137.137	192.192	F
89_ACC_03a	140.156	207.207	172.186	157.163	229.235	109.127	137.139	132.132	206.214	137.137	192.192	F

Table 12. – Multilocus genotypes detected by incidental sampling in the same sampling occasion (13 Sept 2014) when F05 was sampled. F05 was known to be part of a family unit with 3 cubs in the same general area, as from a direct observation dated 11 Sept 2014. Bear 89_ACC_03a is consistent with one of F05's cubs, in that it shares at least one allele at each locus.

Recapture rates by rub-tree sampling and hair-snagging were male-biased (1 female every 3 and 2 males, respectively), whereas they were female-biased by opportunistic sampling at buckthorn patches (Table 13). However, based on the total number of genotypes (n=44) detected by all sampling methods, we computed an empirical female-biased sex-ratio (1:1.20 MM:FF) in the sample, although it varied according to sampling method (Table 13). For reference, the sample and population sex-ratios we estimated in the 2011 survey were 1:1.25 and 1:1.22 MM:FF, respectively.

Sampling method	Samples			Genotypes		
	Females	Males	Sex-ratio (MM:FF)	Females	Males	Sex-ratio (MM:FF)
HS	33	59	1:0.56	11	12	1:0.92
RT	24	64	1:0.38	11	11	1:1
OPP	32	20	1:1.60	6	7	1:0.86
INC	22	22	1:1	11	8	1:1.38
<i>total</i>	<i>111</i>	<i>165</i>	<i>1:0.67</i>	<i>24</i>	<i>20</i>	<i>1:1.20</i>

Table 13. – Recapture rate by sex (samples), and empirical sex-ratio (genotypes) based on hair samples collected in the bear population in the PNALM (June – September 2014) and sampling method (HS: systematic hair-snagging; RT: rub-tree sampling; OPP: opportunistic sampling at buckthorn patches; INC: incidental sampling).

In addition to samples collected from living bears, we also analysed tissue and hair samples from 4 dead bears (see Table 9). Only 2 of these revealed high-confidence scores, namely OrneC0302 and Ornsec0114 (see Appendix B). The first, found dead in 2002, was a 0-MM with ISPra Gen1.13, accordingly sampled only until that year; the second had never sampled before and expectedly so as it was a 2014 cub found dead from unknown causes in August 2014.

² Care should nevertheless be taken in using this as a criterion to distinguish alleged offsprings of known parents. In fact, based on the 78-bear dataset of genotypes scored by WGI, other 19 different genotypes share an allele at all loci with F10 (D. Paetkau, pers. comm). Due to the low genetic variability in this bear population, this points out the inherent limits of such approaches to infer parental relationship within this small bear population.

3.2.4 Genotypes detected by rub-tree sampling

In total, 22 genotypes were detected by rub-tree sampling, with a sex-ratio of 1:1 MM:FF, corresponding to 45.8% and 55% of all females and males, respectively, detected by all sampling methods. Out of 88 successfully genotyped rub-tree samples, 4 (4.5%) revealed to be replicates (i.e., 2 samples collected on the same RT and sampling occasion and left by the same bear). Excluding these replicated samples, 73.8% (n=62) of the remaining 84 rub-tree samples were left by 11 males, at an average rate of 5.6 (± 5.7 SD) samples/male, whereas the remaining 26.2% (n=22) were left by 11 females at a mean rate of 2 (± 1.2 SD) samples/female (Fig. 15). The majority of males (72.7%) and females (81.8%) sampled at rub trees were bears already sampled during previous surveys, and therefore at least 4 years old. Five out of the 11 detected males accounted for 82.3% (n=51) of the male samples, corresponding to 5–19 samples each, while the others males were sampled with 1-3 samples each (Fig. 15a).

Based on the 9 rub trees monitored by camera-traps for which we obtained video clips (n=48) portraying bears, in 7 out of 10 video-captured rubbing events (July – September) we were able to associate the collection of the corresponding hair samples; these were collected from 1 to 12 days after the bear was filmed (Table 14). In 2 of such cases, the rubbing bear portrayed in the clip was individually recognized as previously known bear F09, corresponding to the genotype scored from the hair. The remaining 5 cases involved unknown bears (no obvious presence of collars or other marks). In addition to actual rubbing, we also video-clipped bears displaying different behaviour at rub trees, including investigation (Table 14).

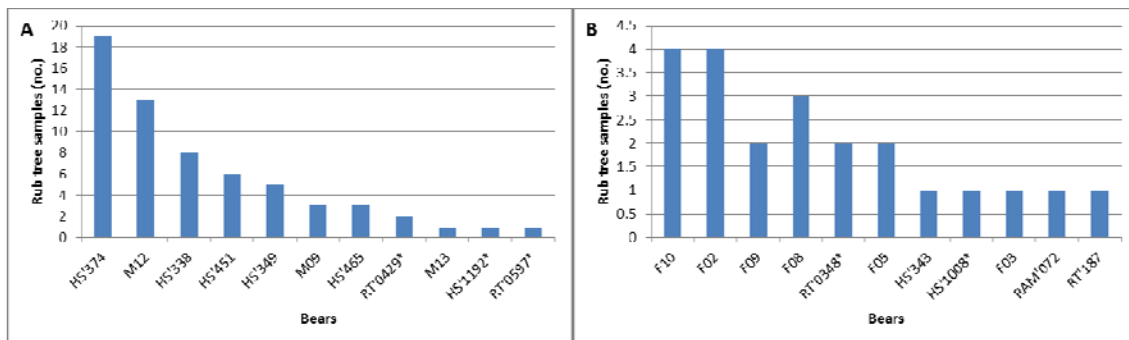


Figure 15. – Distribution of unduplicated rub-tree samples (n=84) collected from (A) 11 males (n=62 samples) and (B) 11 females (n=22 samples) based on the 22 genotypes detected through rub tree sampling (displayed on the x-axis) (PNALM, June – September 2014).

3.2.5 Genotypes detected by incidental sampling

Concerning incidental sampling, 43 (61.4%) of the samples (n=70; cf. Table 10) were collected during verification of alleged damages caused by bears, 32 of which provided genotypes of 12 different bears detected from 1-8 sampling occasions (Table 15; Fig. 16). All of these bears had been already detected during previous surveys. These included one bear known to be regularly problematic (FP01), two bears known to occasionally feed on cultivations (F07, F09), one bear already detected at a damage site during the 2011 survey (ACC079), and one bear whose genotype is compatible with FP01's son (Table 16). Whereas these results have relevant management implications, they also indicate that our final estimate of population size does comprise problem or 'management' bears, or otherwise bears whose home ranges are closer to human settlements. Other 7 bears were detected from samples collected incidentally to other patrolling and field activities, and only one of these (bear HS_349) has been also detected while verifying damages (cf. Fig. 16).

Rub tree code	Clips				Hair samples	Genotype
	Date	hrs	Bear	Behaviour		
158	26 July	13.37	F09	Rubbing	si	no
158	2 Aug	12.56	F09	Investigating	no	
158	18 Aug	15.04	F09	Rubbing	si	F09
158	3 Sept	16.48	F11	Rubbing	no	
83bis	30 July	8.36	unmarked	Investigating	si	HS'451
80	6-12 Aug		unmarked	Investigating	no	
80	29 Aug	1.36	unmarked	Investigating	si	HS'338
80	19 Sept	19.01	unmarked FWC (2 cubs)	Investigating and rubbing	no	
118	10 Aug	18.02	unmarked	Investigating	no	
118	29 Aug	05.09	unmarked	Rubbing	si	no
118	27 Sept	21.45	unmarked male	Investigating and rubbing	si	HS'349
90	8 Aug	22.55	unmarked	Rubbing	si	HS'1008
180	19 July	11.42	unmarked FWC (2 cubs)	Investigating and rubbing	si	RT'0597
75	28 July	23.58	unmarked	Rubbing	si	HS'374
75	11-14 Aug		unmarked	Investigating	si	M12
75	28 Aug- 1 Sept		unmarked male	Investigating	no	
75	26 Sept	18.18	unmarked	Passing by	no	
75	05 Sept	17.10	F05	Passing by	no	
149	18 Aug	21.39	unmarked	Passing by	no	
149	10 Sept	21.39	unmarked	Passing by	si	M13
149	18 Sept	06.46	F10	Investigating	si	F10
149	20 Sept		F10	Investigating and rubbing	no	

Table 14. – List of the 48 video clips (Multipir and Keep-guard IR cameras) portraying bears at 9 rub trees (PNALM, 17 July – 6 October 2014) and corresponding collection of hair samples for genotype identification. More than one clip was obtained in a given date at a given rub tree.

Bear Code	Sex	No. samples	Damages to	Problem bear?
HS338	M	8	livestock	
FP01*	F	7	poultry	known
F13	F	4	crops	
HS343	F	2	livestock	
HS349	M	2	cherry trees	
M09	M	2	livestock	
HS465	M	2	beehives and poultry	genotype compatible with FP01's son
ACC79*	F	1	livestock	
F09	F	1	crops	occasional
HS355	M	1	crops	
RT148	M	1	bee hives	
F07	F	1	crops	occasional

Table 15. – List of the 12 bears detected from 32 hair samples collected during verification of damages to crops, beehives and livestock made by bears. Out of 43 samples collected during damage verification, 11 (25.6%) did not yield DNA (PNALM, June – September 2014). Bears marked (*) are those already sampled at damage sites during the 2011 survey.

Genotype	G10B	G10C	G10D	G10L	MSUT-2	MU59	REN144 A06	CXX20	MU50	MU51	G10X	MU05	MU11	G10P
FP01 (F)	140.156	203.203	172.172	163.163	195.203	229.235	109.109	135.137	132.136	206.214	129.135	137.137	188.192	145.157
HA_465 (M)	140.140	203.207	172.186	163.163	203.203	229.235	109.127	135.137	132.132	206.206	129.129	137.137	192.192	157.157

Table 16. – FP01 multilocus genotype compared to that of HS_465s. Both bears had been incidentally detected during verification of alleged damages to beehives and poultry farms (PNALM, June – September 2014). Bold scores in HS_465's genotype at each marker indicate alleles in common with FP01, revealing it is compatible with FP01's son.

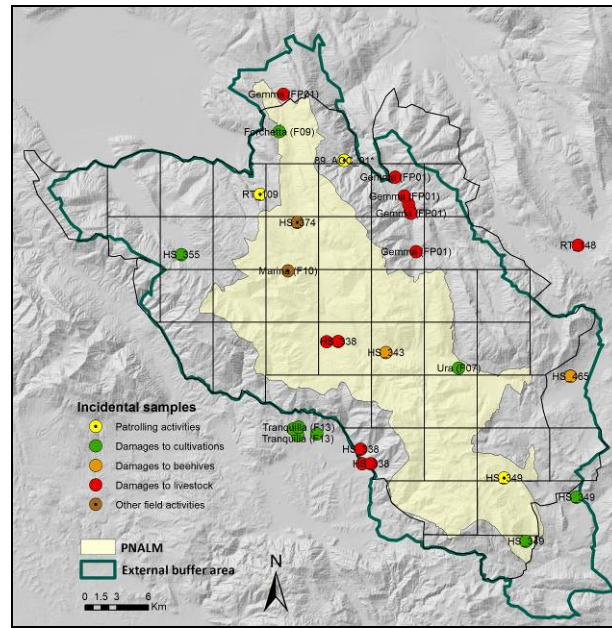


Figure 16. – Incidentally collected bear samples (n=44) that yielded the reliable genotypes of 19 bears (labels) according to type of field inspection (i.e., verification of alleged damages, patrolling, and other field activities; PNALM, June – September 2014). Of these bears, 12 were involved in damages to cultivations, beehives, and livestock. The 2014 systematic sampling grid is overlaid as a reference.

3.3 Modelling

3.3.1 Data sources and encounter history

Out of the 43 noninvasively detected bears used for CR modelling, 22 (51.2%) were sampled by only one sampling technique, 12 (27.9%) by two, 8 (18.6%) by three, and one (2.3%) by all four sampling techniques (Appendix C).

3.3.2 CR modeling

The model selection procedure revealed a significant interaction between a bear's sex and previous hair-snag event (*prev.hs*) on the probability to be detected by hair-snag (model 1 in Table 17). In particular, males with previous hair-snag detection had a higher capture probability than all other bears of both sexes (Fig. 17). In this and in all data types, the effect of the binary variable *prev.hs* was more supported than that of the absolute number of earlier hair-snag detections (*n.hs*). A temporal variation in capture probability for the hair-snag sampling was also supported by the data. Such variation was best described by a negative trend, with mean capture probability decreasing from about 0.22 (95% CI = 0.14-0.34) in session 1 to about 0.09 (95% CI = 0.04-0.16) in session 5 (Fig. 17). None of the spatial variables tested on the hair-snag data (distance from the grid edge and distance from the central mountain ridge) was supported by the data.

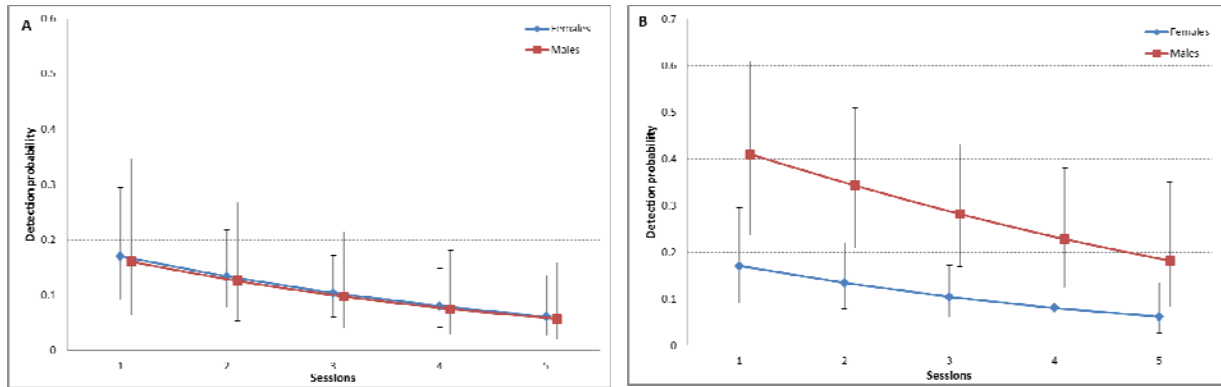


Figure 17. – Capture probability estimates (\pm 95%CI) at the hair-snag sampling for bears with no previous hair-snag detection (A), and bears with previous hair-snag detections (B). Data from the Apennine bear population noninvasive survey in the PNALM, June – September 2014. Capture probability estimates are derived from the most supported model (model 1 in Table 17), and provided separately for the different sexes. Scale on the y-axis differs between panels.

The buckthorn sampling data also supported temporal variation in capture probability (model 1 in Table 17) with a decreasing effectiveness during the sampling session. However, when coupled with the effect of the varying sampling effort, the overall capture probability at buckthorn patches had a bell shape, with the highest values in sessions 2 and 3 (Fig. 18). Also for this data type, the best supported model included an interaction between sex and previous hair-snag detections. Differently from hair-snag, in this case it was females with a previous hair-snag detection which showed higher p than males (Fig. 18b). An additive and negative effect of latitude was also supported by the data, showing that in average buckthorn sampling was more effective in the southern than in the northern part of the study area. Also, there was a strong effect of the distance of each bear's sampling centre from the closest buckthorn patch, with capture probability decreasing at about zero for bears having their sampling centre at more than 3 km from the closest buckthorn sampling area (Fig. 19). None of the other spatial variables tested on the buckthorn data (distance from the grid edge and distance from the central mountain ridge) was supported by the data.

Although a temporal variation in detectability existed for rub tree sampling, this was better explained using the cumulative effort per session (n. of rub tree nights per session) than using a simple time effect or a trend effect (model 1 in Table 17). A strong support was provided to the interaction between sex and a previous hair-snag event. Capture probability estimates from the most supported model show that previously hair-snagged males had a very high capture probability, on average equal to 0.33 (95% CI = 0.22 – 0.47), whereas never hair-snagged males (very likely to be younger than 4 years) had a much lower capture probability, on average equal to 0.04 (95% CI = 0.01 – 0.10) (Fig. 20). Such a marked difference was instead not observed between previously hair-snagged and never hair-snagged females ($p=0.07$; 95% CI = 0.03 – 0.13, and $p=0.08$; 95% CI = 0.03 – 0.15, respectively; Fig. 20). This confirms that the use of rub-trees was proportionally more frequent for males in reproductive age compared to other bears. We also revealed an effect due to the number of rub trees in each bear's "home range", allowing modelling the additional individual heterogeneity, generated by the spatial and temporal variation in the RT sampling effort (Fig. 21). There was no support in the data for an effect of the distance from the grid edge, or from the central mountain ridge, on detection probability at rub trees.

Model No.	Description	AICc	ΔAICc	Weight
1	HS(trend+sex*prev.hs) OPP(trend+lat+sex*prev.hs+effort+ram.dist) RT(effort+sex*prev.hs+nrub) INC(prev.hs+town.dist)	622.51	0.00	0.21
2	HS(trend+sex+prev.hs) OPP(trend+lat+sex*prev.hs+effort+ram.dist) RT(effort+sex*prev.hs+nrub) INC(prev.hs+town.dist)	622.60	0.09	0.20
3	HS(trend+sex+prev.hs) OPP(trend+lat+sex+prev.hs+effort+ram.dist) RT(effort+sex*prev.hs+nrub) INC(prev.hs+town.dist)	623.43	0.91	0.14
4	HS(trend+sex+n.hs) OPP(trend+lat+sex*prev.hs+effort+ram.dist) RT(effort+sex*prev.hs+nrub) INC(prev.hs+town.dist)	623.96	1.45	0.10
5	HS(trend+sex*prev.hs) OPP(trend+lat+sex*prev.hs+effort+ram.dist) RT(effort+sex*prev.hs+nrub) INC(n.hs+town.dist)	624.46	1.94	0.08
6	HS(trend+sex+prev.hs) OPP(trend+lat+sex*prev.hs+effort+ram.dist) RT(effort+sex*prev.hs+nrub) INC(prev.hs)	624.52	2.01	0.08
7	HS(trend+sex+prev.hs) OPP(trend+lat+sex*n.hs+effort+ram.dist) RT(effort+sex*prev.hs+nrub) INC(prev.hs+town.dist)	625.25	2.74	0.05
8	HS(trend+sex+prev.hs) OPP(trend+lat+sex*prev.hs+effort+ram.dist) RT(effort+sex*prev.hs+nrub) INC(null)	625.31	2.80	0.05
9	HS(trend+sex+prev.hs) OPP(trend+lat+sex*prev.hs+effort+ram.dist) RT(effort+sex*prev.hs+nrub) INC(sex)	626.86	4.35	0.02
10	HS(trend+sex+prev.hs) OPP(trend+lat+sex*prev.hs+effort+ram.dist+grid.dist) RT(effort+sex*prev.hs+nrub) INC(null)	627.08	4.57	0.02
11	HS(trend+sex+prev.hs) OPP(trend+lat+sex*prev.hs+effort+ram.dist) RT(effort+sex*prev.hs+nrub+grid.dist) INC(null)	628.95	6.44	0.01
12	HS(trend+sex+prev.hs) OPP(trend+lat+sex*prev.hs+effort+ram.dist) RT(effort+sex*prev.hs+nrub+sex) INC(null)	629.00	6.48	0.01
13	HS(trend+sex+prev.hs) OPP(trend+lat+sex*prev.hs+effort+Log(ram.dist)) RT(effort+sex*prev.hs+nrub) INC(null)	629.09	6.58	0.01
14	HS(trend+sex+prev.hs) OPP(trend+lat+sex*prev.hs+effort+ram.dist) RT(effort+sex*n.hs+nrub) INC(prev.hs+town.dist)	629.16	6.65	0.01
15	HS(trend+sex+prev.hs) OPP(trend+lat+sex*prev.hs+effort) RT(effort+sex*prev.hs+nrub) INC(prev.hs)	634.97	12.45	0.00
16	HS(trend+sex+prev.hs) OPP(trend+lat+sex*prev.hs+effort) RT(effort+sex*prev.hs+nrub) INC(null)	641.38	18.87	0.00

Table 17. – Model selection results for the Huggins closed population estimation, applied to the 2014 survey data of the Apennine brown bear population in the PNALM, Italy. Abbreviations for the data sources indicate hair-snag (HS), opportunistic sampling at buckthorn patches (OPP), rub-trees (RT), and incidental samples (INC). Parameter abbreviations indicate the number of active rub trees in each bear home range (*nrub*), a previous hair-snag detection between 2003 and 2011 (*prev.hs*), the latitude of each bear's sampling centre (*lat*), the distance of each bear's sampling centre to the closest buckthorn patch (*ram.dist*), to the closest village (*town.dist*), and to the border of the sampling grid (*grid.dist*). The models shown are the 16 most supported ones, sorted by AICc values. Several other models have been fitted, which received a negligible support from the data (not listed).

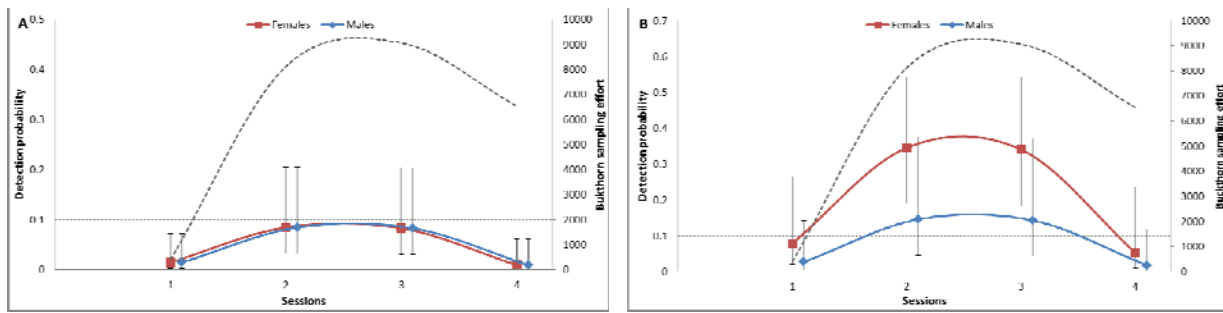


Figure 18. – Capture probability estimates (\pm 95% CIs) through buckthorn sampling for bears with no previous hair-snag detection (A), and bears with previous hair-snag detections (B). Data from the Apennine bear population noninvasive survey in the PNALM, June – September 2014. Capture probability estimates are derived from the most supported model (model 1 in Table 17), and provided separately for the different sexes. Dotted line represents sampling effort (see text). Scale on the y-axis differs between panels.

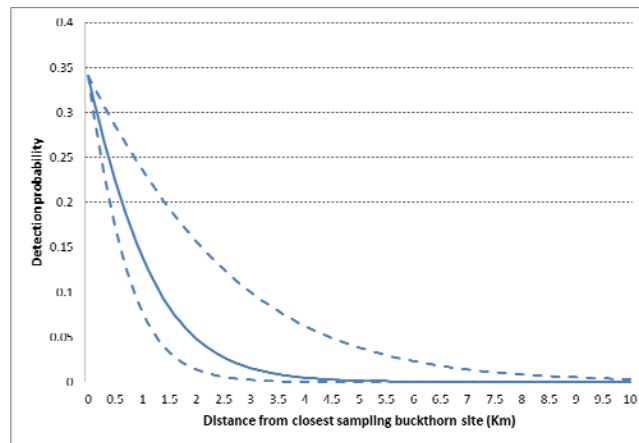


Figure 19. – Relationship between detection probability (\pm 95% CIs) at buckthorn sites and the distance of each bear's sampling centre to the closest buckthorn patch (Apennine bear population noninvasive survey, PNALM June – September 2014). Capture probability estimates are derived from the most supported model (model 1 in Table 17).

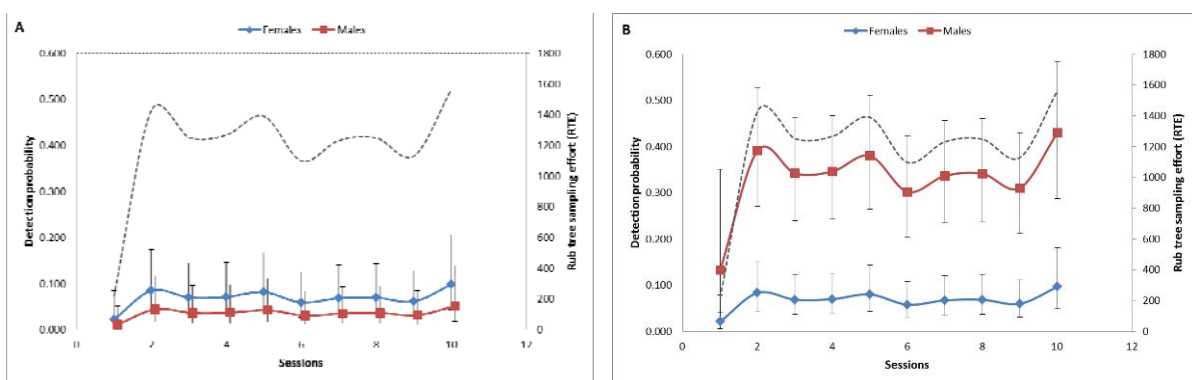


Figure 20. – Capture probability estimates (\pm 95% CIs) for rub tree sampling for bears with no previous hair-snag detection (A) and bears with previous hair-snag detections (B). Data from the Apennine bear population noninvasive survey in the PNALM, June – September 2014. Capture probability estimates are derived from the most supported model (model 1 in Table 17), and provided separately for the different sexes. Dotted line represents sampling effort (see text). Scale on the y-axis differs between panels.

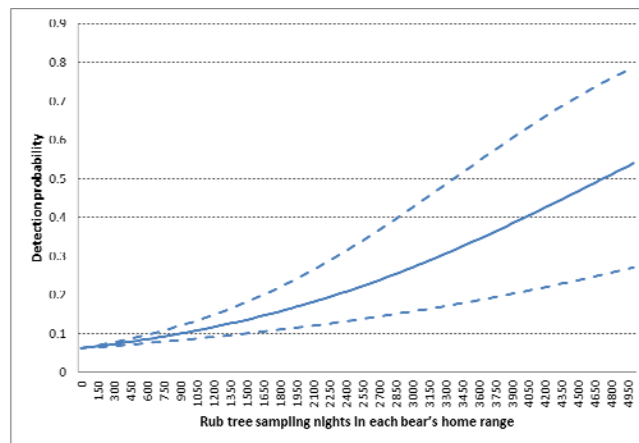


Figure 21. – Relationship between the number of rub tree sampling nights in each bear’s home range and detection probability ($\pm 95\%$ CIs) through rub tree sampling (Apennine bear population survey in the PNALM, June – September 2014). Capture probability estimates are derived from the most supported model (model 1 in Table 17).

When analysing the data derived from incidental samples, no significant difference emerged in capture probability between male and female bears. Instead, a strong effect of a previous hair-snag detection was supported by the data, with previously hair-snagged bears of both sexes exhibiting a higher capture probability ($p=0.50$; 95% CI = 0.31 – 0.70) relative to never hair-snagged bears ($p=0.23$; 95% CI = 0.10 – 0.44). Also, bears living farther from villages and human settlements exhibited a reduced capture probability (Fig. 22).

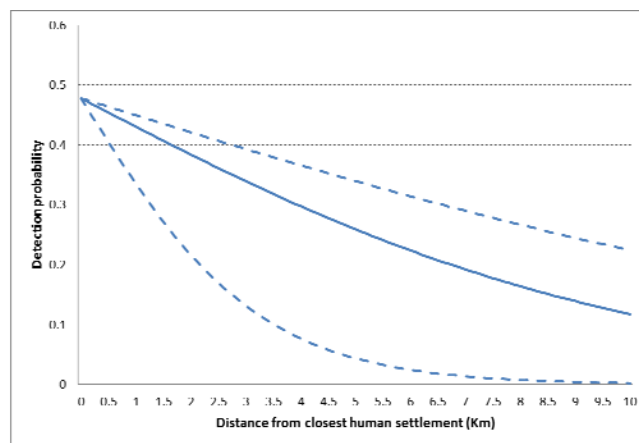


Figure 22. – Relationship between detection probability ($\pm 95\%$ CIs) through incidental sampling and the distance of each bear’s sampling centre to the closest human settlement (Apennine bear population survey in the PNALM, June – September 2014). Capture probability estimates are derived from the most supported model (model 1 in Table 17).

A graphical summary of the group-specific variation in capture probability along all the sampling sessions is depicted in Figure 22. By calculating the probability of being detected in at least one sampling session for each of the four groups shown in Fig. 23, we estimated that the 2014 sampling allowed us to detect 95% of females with previous hair-snag detection, 83% of never hair-snagged females, 75% of never hair-snagged males, and 100% of previously hair-snagged males.

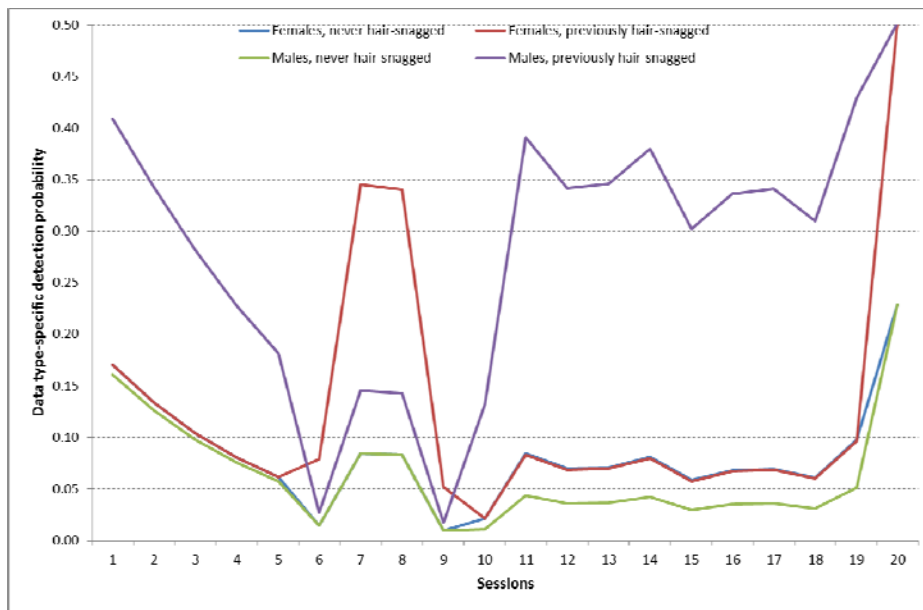


Figure 23. – Variability of capture probability across sampling sessions (Apennine bear population noninvasive survey, PNALM June – September 2014). Sessions 1-5 refer to hair-snag sampling, 6-9 to buckthorn sampling, 10-19 to rub trees, and session 20 to incidental sampling. Capture probability estimates are derived from the most supported model (model 1, Table 17), and provided separately for the different sex and history of previous hair-snagging. Other covariates included in the model (effort, n.rub) have been fixed at their average value.

3.3.3 Population estimate and assessment of the sampling strategy

Based on the above results (see § 3.3.2), and performing a model averaging among all models (Table 17), we produced a final superpopulation size estimate of 50 bears (95% CI = 45-69; CV = 10.5%), including cubs, and corresponding to 22 (95% CI = 20-32) males and 28 (95% CI = 25-37) females. The estimated population sex-ratio was 1:1.27 MM:FF (95% CI = 1:0.78 – 1.85 MM:FF), and the closure-corrected density estimate, based on a previously estimated bear fidelity to the sampling grid of 95.1% (Gervasi et al. 2012), was 38.8 bears / 1000 km² (95% CI = 35.1 – 53.6 bears / 1000 km²).

When compared with all possible reduced designs, the full sampling design likely provided the best balance between accuracy and precision. While we cannot properly assess accuracy (as we do not know true population size), we know that at least 43 bears were present in the superpopulation sampled in our sampling grid. We can therefore assess the risk of underestimating population size with some of the reduced designs. Three of the reduced designs (hair snag + buckthorn, buckthorn + incidental; buckthorn + rub trees) provided estimates of population size within $\pm 10\%$ of the estimate derived from the full design (Table 18, Fig. 24). All the other reduced designs provided estimates at least 10% lower than the one obtained with the full design, and several of them provided estimates lower than the minimum number of bears known to be alive in the study area. When comparing designs in terms of precision, the full one exhibited the lowest coefficient of variation (10.5%). Interestingly, the three designs which provided estimates close to the full one were also the ones with the lowest associated precision, from 20.5 up to 35.2% CV. As we recommended based on the simulations based on the 2011 dataset, the full design was therefore the only one providing an acceptable level of accuracy and good precision of the estimates.

Design	No. bears detected	Pop. size estimate	SE	CV	LCI	UCI
Full design	43	50	5.25	11	45	70
Hair snag + rub trees	32	36	3.93	11	33	52
Rub trees + incidental	30	38	8.01	21	32	73
Hair snag + incidental	31	44	10.64	24	34	84
Buckthorn	13	18	4.57	25	14	36
Rub trees	22	29	8.25	28	23	66
Hair snag	23	34	10.23	30	25	75
Hair snag + buckthorn	32	55	20.57	37	37	135
Buckthorn + rub trees	30	51	21.46	42	34	140
Buckthorn + incidental	28	52	35.23	68	32	148

Table 18. – Population size estimates for the Apennine brown bear population as estimated by CR modelling based on recapture rates as from a noninvasive survey conducted in the PNALM, June – September 2014. Different sampling scenarios are listed, from the full design (hair snag + rub trees + buckthorn + incidental sampling) in the first row, to progressively reduced sampling designs resulting from different combinations of the 4 sampling techniques (see also Fig. 24).

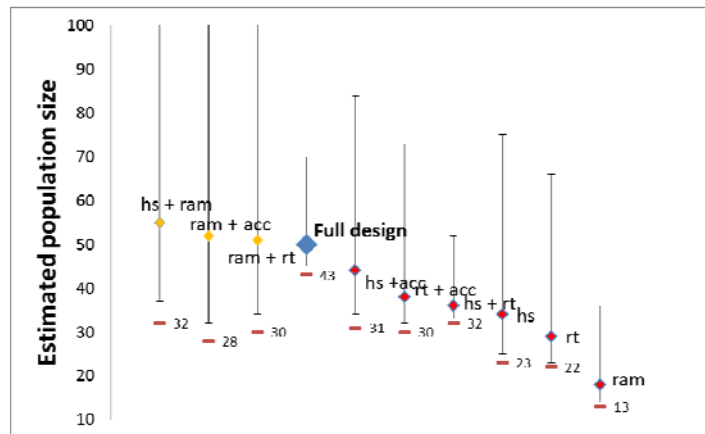


Figure 24. – Population size estimates (\pm 95% CIs) for the Apennine brown bear population as estimated by CR modelling based on recapture rates as from a non-invasive genetic sampling in the PNALM (June – September 2014) and provided by all possible sampling designs, resulting from different combinations of the 4 sampling techniques (hair-snag, buckthorn, rub trees, incidental). Red horizontal tick marks are the number of detected bears with each sampling design.

3.4 Complementary sources of demographic data

In addition to the estimation of the population size in 2011 and 2014, we collected additional demographic data on an annual basis to aid a biologically more meaningful interpretation of the perceived trends in population size across the years of the Arctos project. These additional data sources included: (a) annual counts of female with cubs, as a measure of productivity, and (b) the number of bears retrieved dead, as a measure of minimum detected mortality and mortality causes.

3.4.1 Unduplicated counts of females with cubs: 2011-2014

From 2011 to 2014, each year we estimated the minimum number of family units in the core population according to field protocols we are implementing in the PNALM since 2006 (Ciucci et al. 2009). Results of these counts have been annually distributed among Arctos partners (2011: Ciucci et al. 2011a; 2012: Ciucci et al. 2012; 2013: Tosoni et al. 2013; 2014: Tosoni et al. 2014) and publicly disseminated through the Arctos web site (<http://www.life-arctos.it/documenti.html>). Overall, the minimum number of females with cubs varied from 1 (2011) to 5 (2012 and 2014), corresponding to

3 – 11 cubs produced each year, or a 4-year cumulative sum of 31 cubs (7.8 ± 4 SD cubs/year) (Table 19).

Year	Vantage points	Survey areas		No of operators ^a	Observation effort ^b	Bear sightings/ 100 hrs		Unique no. of FWC ^{c, d}	Cumulative no. of cubs
		N	Km ²			bears	FWC ^c		
2011	60	27	117	19-52	809	15.1	0.4	1	3
2012	56	25	95	44-65	888	14.0	1.7	5	11 ^e
2013	46	25	94	58-78	1636	8,8	0	4 ^f	6
2014	60	24	95	62-74	931	9.7	1.9	5	11 ^g

^a: operators simultaneously active in the single observation sessions (min – max)

^b: limited to simultaneous observation sessions

^c: females with cubs

^d: including also those accounted for by opportunistic observations

^e: of these, observed by the end of July, only 8 have been confirmed alive throughout the end of September

^f: of which 3 directly accounted for during the unduplicated counts of 2013, and 1 added the following year as this female with yearlings was observed for the first time in 2014 having apparently escaped observation in 2013

^g: one cub reported dead on Aug 28, 2014

Table 19. – Sampling effort (direct observations) and corresponding annual productivity estimated for the Apennine bear population in its core range from 2011 to 2014. The technique of unduplicated counts were used annually to detect the minimum number of females with cubs (FWC), females with yearlings (not reported in the table), and the total number of cubs produced. See Ciucci et al. (2011, 2012) and Tosoni et al. (2013, 2014) for further details.

3.4.2 Minimum detected mortality: 2011-2014

As the Veterinary Service of the PNALM maintains an updated database of all bears found dead (L. Gentile, pers. comm., <http://www.parcoabruzzo.it/pdf/orsi.morti.pdf>), we used those data to quantify minimum known mortality during the years the project Life Arctos was conducted. According to these data (Table 20), 12 bears were retrieved dead from 2011 through 2014, including a minimum of 3 females in reproductive age. Mortality causes accounted for shooting, disease (one case of possible pseudorabies and one case of bovine tuberculosis), vehicle accidents and other unknown causes (Table 20). Excluding 2 bears that died in the peripheral portions of the range, and assumed to be demographically independent from the core population that we surveyed during the Arctos project, minimum mortality levels correspond to 2.5 bears/year, which is comparable to what reported during the previous years (1970 – 2009: 2.5 bears/year; Ciucci et al, in prep. L. Gentile, pers. comm.). In addition, assuming dead adults of unidentified sex (n=3) reflect the same proportions of the dead adult bears who have been sexed, we estimated that a minimum of 4 adult females died in the core population from 2011 to 2014, corresponding to 1 adult female bears/year, compared to 0.88 adult female bears/year from 1970 – 2010 (L. Gentile, pers. comm.).

Mortality causes	Adult			Cubs	Total
	FF	MM	Uns.		
poaching		2	1		3
vehicle	2	1 ^a			3
disease	1	1 ^a		1	3
unknown		1	2		3
Total	3	5	3	1	12

^a: these 2 bears have been retrieved dead outside the PNALM ecosystem where the noninvasive survey has been conducted. They refer to an adult male killed by a vehicle on the A24 highway on April 2013, and an adult male live-trapped in the Velino-Sirente National park in 2012 as it was displaying obvious sign of Aujeszki's disease, and that died overnight (for further details see Forconi et al. 2014).

Table 20. – Summary statistics of 12 Apennine brown bears found dead during the years of the Life Arctos project (2011 – 2014). They represent an index of minimum known mortality, as it is likely that other dead bears were not found or retrieved. All bears were retrieved in the PNLAM ecosystem unless otherwise specified in the Table notes.

4. DISCUSSION

4.1 Survey evaluation

4.1.1 Overall design and sampling techniques

As it was defined through the empirical assessment of the 2011 survey (Ciucci et al. 2013), the combined use of multiple noninvasive sampling techniques was particularly effective in terms of samples collected and coverage of the various segments of the population also in the 2014 survey of the Apennine bear population. Through the adoption of complementary, noninvasive sampling techniques, we managed to obtain a quite high overall capture probability of 0.86 (95% CI = 0.62 - 0.95), slightly higher than that reported for the 2008 survey ($p=0.82$; 95% CI = 0.63 - 0.89), but lower than the one associated to the 2011 effort ($p=0.91$; 95% CI = 0.74 - 0.95). However, in 2014 we obtained a per-capita average probability per session ($p=0.146$; 95% CI = 0.122 - 0.173) that was lower both relative to 2008 ($p=0.311$; 95% CI = 0.216 - 0.438) and to 2011 ($p=0.201$; 95% CI = 0.172 - 0.230).

Similarly to the 2011 survey, through the integration of four different sampling techniques we significantly enhanced the detectability of all bears in the population, irrespective of their being marked or not, thereby increasing capture probability of unmarked bears with respect to the 2008 survey (Gervasi et al. 2012). Accordingly, in 2014 we did not have to recur to sighting data of marked bears, as it was originally done in 2008, because the other data sources corroborated individual encounter histories (Appendix C), thereby preventing us from the risk of overestimating the capture probability of unmarked bears or to stretch model assumptions (e.g., lack of correlation among data sources). Hair-snagging provided a lower overall performance with respect to the 2008 survey but not to the 2011 survey. Whereas this confirms that a process of overall habituation by previously hair-snagged bears may be occurring in our small bear population through repeated surveys (Ciucci et al. 2013), it also suggests that the use of secondary lures that we made in 2014 may have halted a further decrease in the attractiveness of the main lure to bears. As such, the use of secondary lures from the second hair-snag session onward has to be included in further systematic surveys of this bear population.

Based on these results, we therefore confirm that the sampling design we used in 2011 and 2014, although logistically more complex, is appropriate for this small-sized bear population, as it increases sampling coverage, provides a larger sample size and is theoretically more robust, while not implying previous live-trapping and presence of marked bears in the population. Whereas hair-snagging ensures a theoretically complete coverage of the study area, other complementary sampling methods enhance sampling coverage for those segments of the population hardly or more difficult to sample by hair-snagging alone. Buckthorn sampling appears to be particularly successful for females and family units, including cubs, whereas incidental sampling detected bears in more peripheral areas some of which prone to cause damage to farms and crops. However, in 2014 buckthorn sampling proved less successful compared to 2011, contributing the most to the lower number of samples analyzed in 2014 relative to 2011 (Table 21). This was quite an unexpected result, as in 2014 we added 2 main buckthorn areas to the overall sampling scheme relative to 2011. We have no indications as to why this may have happened, even though these findings confirm annual trends in bear sightings at buckthorn area (Tosoni et al. 2014) and somewhat corroborate our previous hypothesis, based on food habits data, that *Rhamnus* patches may be becoming less and less attractive to bears in recent years (Ciucci et al. 2014). Conversely, in 2014 we confirmed that rub tree sampling is essential to enhanced recapture rates for both males and females, and contributes significantly to increase their capture probability and hence to the precision of the final population size estimate. However, based on the 2011 results, in 2014 we tried to enhance efficiency of rub tree sampling. On one hand, we did not collect uninformative replicated samples (i.e., samples left by the same bear on the same rub tree in the same rubbing event) and, on the other hand, we expanded the spatial coverage of sampled rub trees within the survey area while subsampling those

already available in densely-sampled areas (see § 2.2.2). As a net result, the number of samples collected through this sampling technique has been 21.2% lower relative to 2011 (Table 21), though providing a comparable number of genotypes, each at a 48.5% lower cost for genetic analyses (Table 22). The higher efficiency of the 2014 sampling compared to 2011 is also apparent by the overall lower costs for genetic analyses per detected genotype and, more importantly, the higher ratio of genotypes per analyzed sample (Table 22). However, it should be also taken into account that the lower number of markers we used in 2014 also contributed to a higher efficiency with respect to 2011 (Paetkau 2012).

	2011	2014	2011				2014			
			HS	RT	OPP	INC	HS	RT	OPP	INC
Collected	679	476	159	278	139	67	118	207	81	70
<i>to lab</i>	599	466	159	253	122	65	118	204 ^a	75 ^b	69 ^c
<i>replicates not analyzed</i>	28	88	-	27	-	1	-	77	1	10
<i>inadequate</i>	42	32	17	4	17	4	9	7	15	1
Analyzed	529	346	142	222	105	60	109	120	59	58
<i>culled</i>	103	70	40	40	6	17	17	32	7	14
<i>successful</i>	426	276	102	182	99	43	92	88	52	44
% <i>successful</i>	80.5%	79.8%	71.8%	82%	94.3%	71.7%	84.4%	73.3%	88.1%	75.9%

Table 21. – Comparison of the 2011 vs 2014 sampling performance in terms of number of bear hair samples collected and analyzed to detect individual multilocus genotypes (Life Arctos noninvasive surveys of the Apennine bear population in the PNALM ecosystem in 2011 and 2014). Four different sampling techniques have been employed in both surveys (HS: hair-snag; RT: rub tree sampling; OPP: opportunistic sampling at buckthorn patches; INC: incidental sampling).

	2011	2014	2011				2014			
			HS	RT	OPP	INC	HS	RT	OPP	INC
Genotypes	45	44	26	21	22	10	23	22	13	19
Unknown genotypes ^a	16	15	8	4	8	2	7	5	3	3
Unknown/all genotypes (%)	36%	34%	31%	19%	36%	20%	30%	23%	23%	16%
Uniquely detected genotypes ^b	20	21	8	2	8	2	4	5	6	6
Genotypes sampled only once ^c	4	10	4	1	5	6	2	3	2	3
Genotypes/analyzed sample	0.09	0.13	0.06	0.01	0.08	0.03	0.21	0.18	0.22	0.33
Uniquely detected genotypes/ analyzed sample	0.04	0.06	0.06	0.01	0.08	0.03	0.04	0.04	0.10	0.10
Euro/genotype ^f	673	467	325	629	284	357	281	324	270	181

Table 22. – Comparison of the 2011 vs 2014 sampling performance in terms of number of detected, new (i.e., undetected during previous surveys), and uniquely detected bear genotypes (Life Arctos noninvasive surveys of the Apennine bear population in the PNALM ecosystem in 2011 and 2014). Estimates of genetic costs are also provided, based on a total of € 31,499.00 and € 20,599.00 in 2011 and 2014, respectively). Four different sampling techniques have been employed in both surveys (HS: hair-snag; RT: rub tree sampling; OPP: opportunistic sampling at buckthorn patches; INC: incidental sampling).

Comparing the number of collected, analyzed, and successful samples between in the 2001 vs the 2014 surveys (cf. Table 21), not only fewer samples were collected in 2014, but in this year a proportionally lower of such samples were further considered for genetic analyses (i.e., 88.3% in 2011 vs. 74.2% in 2014). The difference mostly accounts for a disproportionate number of samples in 2014 composed of too few hairs or collected without the bulb (D. Paetkau, com. pers.), this possibly being related to a higher number of operators being involved in the collection compared to the 2011

survey. Whereas this difference was not revealed for hair-snagging samples (89.3% vs. 92.4%), it was apparent for all the complementary sampling methods (rub tree: 87.7% vs. 58.8%, buckthorn: 86.1% vs. 78.7%; incidental: 92.3% vs. 84.1% for 2011 vs 2014, respectively). In addition to a lower number of samples collected relative to 2011, a higher proportion of those collected being discarded for genetic analyses in 2014 might have contributed to an overall lower capture probability and a slightly larger coefficient of variation, reflected in 95% confidence intervals slightly larger than those reported in 2011 (Table 23). Although the precision obtained in 2014 is still more than adequate, given the size of the population, for management purposes, this result points out how the actual collection of entangled hair samples represent simple but critical step of the whole survey procedure, especially if the reason for discarding (i.e., not analyzing) the samples is the loss of hair roots or too few of them being collected.

However, based on the number of detected genotypes in 2014, and hence the population size estimate, it is apparent how the complementary adoption of all four sampling techniques (i.e., full sampling design) is the only sampling design able to ensure a higher sample coverage relative to progressively reduce sampling designs, corresponding to an adequate precision for this small bear population and conceivably to more accurate estimates (Table 18). Therefore, even though the full sampling design is costly and logistically more complex, none of the reduced designs corresponds to a positive cost/benefit ratio. The loss of precision (and possibly accuracy) due to the omission of one or more sampling methods would fatally outweigh, in magnitude and consequences, any reduction in costs.

4.1.2 Genetic dataset and marker system

On average, 20.2% of the collected hair samples delivered for genetic analyses failed to produce reliable genotypes, a proportion similar to what obtained in the 2011 survey (19.5%), corresponding to an overall success rate of 79.8%, although this varied according to sampling technique (Table 21). In considering success rates, it is worth noting that WGI has an unusually low tolerance for samples with missing (i.e. low-confidence) data (Paetkau 2003). This is because such data are associated by definition with samples from which it is difficult to amplify all alleles, and thus where the risk of genotyping error (especially allelic dropout) is heightened. Furthermore, missing data increase match probabilities in ways that are difficult to quantify or control. For example, if we settle on a 12-locus system for individual identification, tolerating samples that have low-confidence data for up to 3 markers, we could encounter pairs of samples which are missing data for different subsets of 3 markers, leaving just 6 markers in common between samples for the purposes of deciding whether they came from the same individual. Clearly the associated increase in match probability would undermine the quality of the dataset.

Another consideration regarding success rates is that the relative invariability in the Apennine bear population makes it necessary to be unusually cautious with regards to potentially mixed samples. In a typical population, where one might see 8 or 9 alleles per marker, mixed samples stand out by amplifying 3 or 4 alleles at some markers. Those alleles might differ in strength if the mixture is uneven, but they are still noticeable in cases where the mixture is strong enough to create a risk of false individual identification. By contrast, when there are only 2 or 3 alleles per marker as in the studied population (Ciucci et al. 2013), we cannot rely on this method to identify mixed samples. Thus, we have to be particularly aware of cases where 1 allele in a heterozygous genotype is atypically strong, since this might be a mixed sample where 1 bear was homozygous and the other heterozygous. There is a meaningful risk that mixed samples like these could create chimeric genotypes that are identified incorrectly as unique individuals. Many of the low-confidence scores we revealed in this study represent such imbalances between alleles, and were accordingly culled from the analyses. It was previously estimated that as many as 20% of culled samples in surveys of the Apennine bear population may actually be mixed rather than weak samples (Paetkau 2012).

The final set and number of markers which have been identified and used in this study appears to be optimal for the noninvasive identification of single bears in this population, both because we introduced some new and informative markers with respect to the panel previously used by ISPRA, and because the 9-10 loci in common with the previous lab ensure the comparability of genotypes between labs. To this end, we also produced conversion factors using genotypes scored from blood samples and scored by both labs, and these allow the recombination of the ISPRA and WGI genetic dataset in a unique data base for this population, provided some suspicious cases are further investigated (Ciucci et al. 2012).

As we highlighted at the end of the 2011 survey that we might have used more markers than strictly necessary to achieve a low match probability, and as it is obviously not desirable to analyze more markers than necessary (Waits and Leberg 2004, Paetkau 2004), we used simulations to define an ideal set of markers for the Apennine bear population by: (a) retaining 9 markers in common to both labs, (b) removing markers G10X and MSUT-2 from the larger set ($n=14$) that we tested in 2011, as to enhance efficiency and reduce the chances of genetic errors while retaining adequate information, for a total of 11 markers plus sex; and (c) using an additional marker (G10P) for equivocal matches (i.e., 0-2MM pairs) in comparing current genotypes with those detected before 2011.

As from the 2011 survey, the approach we followed to estimating the probability of sampling more than 1 individual with a given multilocus genotype is to extrapolate from an observed mismatch distribution rather than estimating match probabilities (Paetkau 2003, Ciucci et al. 2015). This empirical approach provides more meaningful insight than calculated match probabilities (i.e., P_{ID} and $P_{ID\ SIB}$), because the distribution of degrees of relatedness among the sampled individuals — an unknown parameter that has a profound effect on calculated match probabilities — is implicit in the mismatch distribution. The current 12-locus dataset of 78 bears (i.e., all bears scored at WGI, including 25 live-trapped between 2006 and 2010, those sampled in 2011 and 2014, and some bears retrieved dead) contains one 1MM-pair, and 3 2MM-pairs (*cf.* Fig. 12). Even with the high consanguinity expected in a population of this size, and despite the low and decreasing variability, matches at all 12 markers are less likely than 1MM-pairs. This suggests that the current marker system is likely to uniquely identify each individual in the sample, and that the risk of false matches within this dataset (i.e., 78 WGI bears) is still well controlled. However, this means that false matches are just unlikely, not necessarily impossible, so it is fundamental to use other field data to check for any unusual matches to be confirmed by analysis of more markers. In addition, due to a rapid loss of genetic variability (see § 4.2) it is fundamental to recognize that additional loss of variation over time could increase the risk of false matches. Even more problematic is the realization that considering all bear genotypes detected from 2000 to 2008 and analyzed by ISPRA (i.e., ISPRA dataset), which are based on 11 markers (10 of the 12 markers used by WGI, plus G10P, which is used by WGI to scrutinize any new genotype that matches a reference sample at the first 10 markers), it becomes obvious that sample size alone dictates that the risk of false matches will be greater. More individual bears in the comparison means more opportunities for false matches, while the reduced number of comparatively variable markers exacerbates this risk. Accordingly, there are 25 2MM-pairs in an 11-locus dataset comprised of 94 bears judged likely to be unique (i.e., ISPRA and WGI datasets combined), versus 3 such pairs in the WGI 12-locus dataset of 78 bears (see above). Thus, the risk of false matches is considerably (~ 8 -fold) greater in comparisons involving a larger number of bears using the 11-locus dataset.

4.2 Population size and trends: the ex-ante ex-post approach

Our estimate of the 2014 population size is practically identical to the size we estimated in 2011, both overall and separately for males and females (Table 23). Contrasting the abundance of the bear population in 2014 with that in 2011 results in a yearly rate of increase of $\lambda = 0.99$, based on point estimates alone, while the respective confidence intervals fully overlap (Table 23). Accordingly, using

the Delta method to estimate the variance about the yearly rate of increase yields 95% CI for λ that ranges from 0.85 to 1.14, where $\lambda = 1$ signifies a stable population from year to year. Similarly, both the population density, corrected for the closure violation, and the population sex-ratio resembles the values estimated in 2011, revealing that no abundance nor major structural changes affected the population during this 4-year period.

Year	N _{tot}		N _{males}		N _{females}		CV%	Sex-ratio (MM:FF)	Bears/ 1000 km ²
	\hat{N}	95% CI	\hat{N}	95% CI	\hat{N}	95% CI			
2011 ^a	51	47 – 66	23	21 – 31	28	26 – 35	7.9%	1:1.22	39.7
2014	50	45 – 69	22	20 – 32	28	25 – 37	10.5%	1:1.27	38.8

^a: this is a slightly different estimate of population size compared to what originally reported (i.e., 49 bears, 95%CI: 47 – 61 bears, CV% = 7%) due to a slightly different specification of the most supported model (cf. Ciucci et al. 2015)

Table 23. – Comparison of the 2011 vs. the 2014 (this study) estimates of the Apennine bear population in the PNALM ecosystem.

The 2014 estimate, as well as the one from 2011, comprises all age cohorts, including cubs of the year. These in 2014 have been estimated by direct observations (i.e., unduplicated counts of female with cubs) at a minimum of 11 distributed 5 family groups (Tosoni et al. 2014). Assuming they represented all cubs in the population, cubs would account for 22% (95% CI: 16 – 24%) of the population in 2014, indicating a quite high reproductive performance for such a small bear population. However, this may not be representative of the average conditions in the population as the number of cubs in 2014 may have been positively affected by the occurrence of a beechnut mast year in fall 2013. Alternatively, by assuming the population remained stable during this 4-year period, and using an average of 7.8 cubs produced each year (cf. Table 19), this would correspond to a mean of 15.6% (95% CI: 11 – 17%) cubs in the population. Conversely, as we estimated that on average 3.75 (± 1.9 SD) females reproduced each year from 2011 to 2014 (Tosoni et al. 2014), or that about 11 adult females were present in the population any given year (i.e., 3.75 females/year * 3 years of inter-birth interval), reproductive females represented on average a minimum of 22.5% (95% CI: 16 – 25%) of the bear population, and 42.2% (95% CI: 30 – 45%) of females of all ages. Although these estimates are obviously to be interpreted as crude and tentative, as different and more reliable approaches should be used to assess population structure, their inherent value rests in the fact that they are the first to be produced for this bear population.

We detected no negative trends from 2011 to 2014 and this, while taking into account sources of sampling and modelling variability, allows us to confirm definitively that the relict Apennine bear population in its core distribution is still reproductively active, demographically capable of positive growth (notwithstanding substantial levels of human mortality), and potentially able to act as a source population. These, per se, can be viewed as a relative measure of success of the Life Arctos project, because such a small and relict bear population might have been otherwise largely susceptible to deterministic and stochastic events that, as previously suggested (e.g., Wilson and Castellucci 2002), would tend to drive the population to extinction. The *ex ante* and *ex post* monitoring phases indicate that the Apennine bear population still features a good reproductive performance, to the extent of being able to counteract the persistently high human-caused mortality. On the other hand, by acknowledging these persistently high levels of human-caused mortality one may challenge the effectiveness of the conservation actions implemented by the Life Arctos. During 2011 – 2014, reported mortality levels (Table 20) were as high or higher than in the previous decades, and were responsible for the removal of a minimum estimate of 5 female bears in reproductive age from the core population. It should be recognized, however, that with few exceptions (e.g., actions C4 and C10), most actions planned in Life Arctos were not meant to reduce

immediate mortality (i.e., poaching, vehicle accidents, disease), as these were assumed to be under the institutional responsibility of authorities locally in charge of wildlife management and conservation. Most of the Life Arctos actions were instead planned and projected to: (a) provide background data to better describe the bear-human context (e.g. actions As), (b) reduce bear-human conflicts (e.g. actions Cs), and (c) enhance tolerance of humans toward bears (e.g., actions Ds). Most of these actions, therefore, would be expected to reveal their effects in terms of bear population not immediately but in the medium and long term. In this context, a more practical and immediate evaluation of the Life Arctos project would benefit from a simple check-list of the planned actions which had been effectively implemented on the ground.

Aside from the considerations above, it may be insightful to speculate why we observed no population increase despite the annual positive productivity that we recorded (*cf.* Table 19). These revealed that, since the 2011 estimate of population size, a minimum of 28 cubs were added to the population from 2012 to 2014 and, even assuming a substantial natural mortality of cubs (e.g., 50%), some cubs would nevertheless have been able to reach adulthood and contribute to an expected increased population size. Two broadly defined scenarios might explain why this did not occur. The first scenario provides that known and unknown bear mortality in the PNALM ecosystem added to unsustainable levels for the bear population to increase further and expand significantly beyond the core range. In this case, most of the reproductive potential would have been wasted to replace losses to human-caused mortality. Had the reproductive performance been lower than what we recorded, the bear population would have been otherwise waning and getting further closer to extinction under the current mortality levels. If we contrast the minimum number of births during 2012-2014 (28 cubs) with the minimum number of bears reported dead (10 bears, limited to the core range), this scenario is realistic and compatible with the estimated population sizes in 2011 and 2014. In fact, by adding 14 cubs (50% mortality rate) to a population of 51 bears in 2011; by assuming all bear cohorts experienced 100% survival from 2 years of age onward; and finally by subtracting the 10 bears retrieved dead in the same period yields an expected population size in 2014 of 55 bears, well within the 95% confidence interval we obtained (Table 23). This scenario would also accommodate for higher mortality rates of cubs, juveniles and subadult bears, still projecting a population size in 2014 compatible with what we estimated. The second scenario entails that the bear population in the PNALM ecosystem might have approached the carrying capacity. Consequently, juvenile and subadult bears would be forced each year to leave the core and disperse into the peripheral portions of the range. Such hypothesis seems to be somewhat supported by increased records of bear presence in several peripheral areas (e.g., Di Clemente et al. 2012, Carotenuto et al. 2014, Forconi et al. 2014), although it is debated if this is effectively due to an increased number of bears in the peripheral areas, or to a higher and better organized sampling effort in these areas. Indeed, only one female with cubs has been reported outside the core range as recently as November 2014 (A. Antonucci, pers. comm.). In practice, the unsustainable mortality vs. the dispersal hypotheses above are not necessarily mutually exclusive, and the demographic pressure of mortality vs. dispersal are expected to fluctuate from year to year, according to stochastic events (i.e., occurrence of human-caused mortality) and/or annual variation in reproductive output (*cf.* Table 19). Human-induced bear mortality is expectedly high also in the peripheral portions of the range (Falcucci et al. 2009, Forconi et al. 2014), where it could depress the expansion of the range, already naturally slow and hampered by the high philopatry of female bears. Clearly, more intensive and individually-based approaches are needed to closely investigate population dynamics inside and outside the core range. In any event, our findings do support the contention that a rapid expansion beyond the core would be most likely facilitated by a more stringent and effective prevention of all human-induced mortality factors both inside and outside the core range (Ciucci and Boitani 2008, Falcucci et al. 2009). In this perspective, the reproductive potential of the Apennine bear core population, or its demographic stability despite the persistently high human-induced mortality levels, are clear indications that, provided adequate prevention of mortality, expansion of the population still is a biological realistic and plausible conservation goal.

Finally, it is noteworthy that the new genotypes detected in 2014 (assuming they mostly represent a new generation) featured a mean observed heterozygosity for the 11 microsatellites that was lower by 0.04 compared to the bears previously (i.e., ≤ 2011) detected. Consistent with a loss of variability between generations in a small population with no immigrants, this represents a rapid loss, suggestive of a very small effective population size (N_e). In addition, 3 alleles that were present in previous genotypes (e.g., allele 154 at marker *G10B*), were missing from the new genotypes, providing another illustration of genetic drift in real time. These findings underline the urgency of immediate, effective, and aggressive conservation actions with the goal to allow a rapid population expansion beyond the current (core) range, as this would be the only practical means to mitigate further genetic erosion, while reducing the risk associated with the only source population being present in only one location.

4.3 Conclusions and management implications

The Apennine bear population in the PNALM ecosystem did not decline nor increased during the years of the Life Arctos project (2011 – 2014). Instead, the population remained stable, apparently due to a positive reproductive output consistent enough to balance persistently high levels of human-caused mortality and/or dispersal beyond the core range. In these conditions human-caused mortality may dramatically reduce the potential of population increase and its expansion beyond the core area, thus compromising the inherent capabilities of the bear population to escape from the high extinction risks due to small numbers. In this respect, it is noteworthy that in 2011 only one adult female reproduced in the population, after 6 adult females were found dead in the previous 4 years (L. Gentile, pers. comm.).

On the contrary, the closure-corrected density we reported for the core Apennine brown bear population lays in the upper tail of brown reported bear densities (e.g., Proctor et al. 2010, Kindberg et al. 2011). This supports the contention that habitat productivity in the PNALM ecosystem is currently adequate for bears (Ciucci et al. 2014) and underlines the role of the PNALM ecosystem as a last, critical stronghold of brown bears in the Apennines. Although no formal estimates of carrying capacity are available for the PNALM (Ciucci and Boitani 2008), this result argues that any long-term conservation strategy of this remnant bear population should envision the expansion of the bear range across the central Apennines, provided appropriate conservation actions are implemented both within and outside protected areas (Boscagli 1999; Ciucci and Boitani 2008; Anonymous 2011). Among these, the aggressive prevention of any human-related mortality factor appears the most important and urgent action, as this would allow the bear population in the PNALM to act as a source population from year to year. Whichever the mechanisms underlying the population dynamics in the core and surrounding areas (see § 4.2), reducing human-caused mortality would increase the availability of young and subadult bears to disperse into the peripheral areas, as well as the demographic and social pressure inside the core range that would force bears to disperse.

Although we did not obtain evidence of inbreeding depression in the Apennine brown bear population, genetic monitoring in this 4-year period at the level of neutral, microsatellite markers empirically revealed the fast pace of genetic erosion due to an extremely small effective population size (N_e). In the absence of other source populations, the expansion of the range over a larger area across the central Apennines appears the only functional conservation solution to save this bear population and avoid further genetic erosion. Whereas the Life Arctos project might have helped addressing bear-human conflicts and refining practical solutions to enhanced coexistence locally (i.e., best practices), these solutions still need to be permanently implemented over a larger scale across the Apennines.

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Appendix A

Estimation of the age-specific probability to have remained undetected to all previous DNA sampling effort and to be still alive in the study area at the beginning of the 2014 sampling.

Prior to 2014, four extensive DNA surveys of the core Apennine brown bear population had been conducted in 2004, 2007, 2008 and 2011 (Gervasi et al. 2008, 2010, 2012, Ciucci et al. in press), and no cubs were sampled through hair-snag during all these surveys. Hence, any bear that at the beginning of the 2014 survey had already been sampled in previous hair-snag surveys was born before 2011. Based on each bear's previous detection history, we therefore used a binary variable (prev.hs: previous hair-snag history) separating bears >4 years old from younger bears (≤ 4 years) in order to account for the potential effect of a bear's age on capture probability. However, age classification is problematic for those bears sampled in 2014 but not before, as these comprise: (a) bears actually born between 2010 and 2014 (i.e., ≤ 4 years old), and (b) bears born before 2010 which survived through 2014 but remained undetected in previous surveys. Clearly, only if probability of the latter is reasonably small we then can use prev.hs as a proxy of age class. To estimate this probability we used the formula:

$$F_{a,g}^* = \{[(1 - P_{2004,g}) \cdot (1 - P_{2007,g}) \cdot (1 - P_{2008,g}) \cdot (1 - P_{2011,g})]\} \cdot \phi_c \cdot \phi_a^{a-1}$$

where $F_{a,g}^*$ is the probability for an individual of age a and gender g to remain invisible to sampling until 2014. Sex specific capture probabilities in the 2004, 2007, 2008 and 2011 surveys were derived from Gervasi et al. (2008, 2010, 2012) and Ciucci et al. (2014), whereas ϕ_c and ϕ_a are yearly survival probabilities for cubs and adults, respectively (P. Ciucci unpublished data), assuming these remain constant across survey years. The complementarity of $F_{a,g}^*$ (%_{corr}; Table S1) provides an estimate of the proportion of bears correctly classified as ≤ 4 years, suggesting that a great majority of bears with no previous hair-snag history are expected to be ≤ 4 years old. This is especially true for male bears (%_{corr} ≥ 0.83), whereas a certain risk of misclassification exists for females of the 2008-2010 cohorts, dropping to 6% for older females.

Year of birth	Females							Males						
	P ₂₀₀₄	P ₂₀₀₇	P ₂₀₀₈	ϕ_c	ϕ_a	P*	% _{corr}	P ₂₀₀₄	P ₂₀₀₇	P ₂₀₀₈	ϕ_c	ϕ_a	P*	% _{corr}
2004	0.31	0.44	0.7	0.58	0.88	0.02	0.98	0.31	0.45	0.96	0.58	0.96	0.01	0.99
2005	0.31	0.44	0.7	0.58	0.88	0.03	0.97	0.31	0.45	0.96	0.58	0.96	0.01	0.99
2006	0.31	0.44	0.7	0.58	0.88	0.03	0.97	0.31	0.45	0.96	0.58	0.96	0.01	0.99
2007	0.31	0.44	0.7	0.58	0.88	0.06	0.94	0.31	0.45	0.96	0.58	0.96	0.01	0.99
2008	0	0.44	0.7	0.58	0.88	0.22	0.78	0	0.45	0.96	0.58	0.96	0.13	0.87
2009	0	0.44	0.7	0.58	0.88	0.23	0.77	0	0.45	0.96	0.58	0.96	0.15	0.85
2010	0	0	0.7	0.58	0.88	0.24	0.76	0	0	0.96	0.58	0.96	0.17	0.83

Appendix B

Multilocus genotypes of the 44 bears noninvasively sampled during the 2014 survey, plus 2 bears retrieved dead (cf. Table 9). Genotypes had been scored using 11 markers plus sex, and plus G10P for equivocal matches with previously detected (i.e., 2003-2008) genotypes. Multilocus genotypes are expressed in WGI scores. Markers highlighted in red are in common with ISPRA (see Ciucci et al. 2013 for conversion factors to translate WGI into ISPRA scores and vice versa) (*Table continues in the next pages*).

Genotype	# loci	G10B	G10C	G1D	G10L	MU59	REN144 A06	CXX20	MU50	MU51	MU05	MU11	G10P	Sex	Matches with ISPRA dataset
89_ACC_01	12	140.140	207.207	186.186	163.163	229.235	109.127	135.137	132.132	206.214	137.137	188.192		F	
89_ACC_03a	12	140.156	207.207	186.186	163.163	229.235	109.127	137.139	132.132	206.214	137.137	192.192		F	
89_ACC_04	13	140.156	203.203	184.186	157.163	229.235	109.127	135.139	132.132	206.214	137.137	192.192	157.157	M	
Acc'079	13	140.140	207.207	172.186	163.163	229.235	127.129	137.139	132.136	206.206	137.137	192.192	157.157	F	Gen1.2
Atessa (F05)	13	140.156	203.207	172.186	157.163	229.229	109.109	137.139	132.132	206.206	137.137	192.192	157.157	F	Gen1.22
Cicerone (M12)	13	156.156	203.207	172.186	157.163	229.229	109.127	135.137	132.132	206.214	135.137	192.192	145.157	M	Gen1.24
Forchetta	13	140.156	203.203	172.186	157.163	229.235	127.127	135.137	132.132	206.214	137.137	192.192	145.157	F	Gen1.74
Gemma (FP01)	13	140.156	203.203	172.172	163.163	229.235	109.109	135.137	132.136	206.214	137.137	188.192	145.157	F	Gen1.7 (Gen1.63: 1MM)
HS'028	13	140.156	207.207	172.184	163.163	235.235	109.129	135.139	132.136	214.214	137.137	192.192	157.157	F	Gen1.50
HS'0853	12	140.140	203.203	172.184	157.163	229.235	109.127	135.135	132.136	206.206	137.137	192.192		F	
HS'0956	12	156.156	203.207	172.186	157.157	229.235	109.127	137.139	132.136	206.212	135.137	188.188		F	
HS'0980	12	140.156	207.207	172.186	157.163	229.235	127.127	137.137	132.132	206.206	135.137	188.192		M	
HS'1008	12	156.156	207.207	172.184	163.163	229.235	109.127	139.139	132.136	214.214	135.137	192.192		F	
HS'1139	13	140.140	203.207	172.186	163.163	229.229	127.129	135.137	132.132	206.214	137.137	192.192	57.157	M	
HS'1192	12	156.156	207.207	172.186	157.163	229.235	127.127	139.139	132.136	206.212	135.137	188.192		M	
HS'1293	13	140.140	203.203	172.184	163.163	235.235	127.127	135.137	132.132	206.206	137.137	188.192	157.157	M	
HS'330	12	140.156	203.203	172.172	163.163	229.235	127.127	135.137	132.136	206.214	135.135	192.192		F	
HS'338	12	140.156	203.207	186.186	163.163	229.229	109.109	135.139	132.132	206.214	135.137	192.192		M	
HS'343	13	140.140	203.207	172.172	163.163	229.235	109.129	137.137	132.136	206.206	137.137	188.188	157.157	F	Gen1.18;
HS'349	13	154.156	203.207	172.186	157.163	229.229	109.129	137.137	132.136	206.206	135.137	192.192	145.157	M	

APPENDIX B
continued

Genotype	# loci	G10B	G10C	G1D	G10L	MU59	REN144 A06	CXX20	MU50	MU51	MU05	MU11	G10P	Sex	Matches with ISPRA dataset
HS'355	13	156.156	207.207	172.172	157.163	229.229	127.129	135.137	132.132	206.206	135.135	188.192	157.157	M	Gen1.76
HS'374	12	140.140	203.207	172.186	157.163	229.229	127.127	139.139	132.136	206.206	135.137	188.192		M	
HS'451	12	140.156	203.207	184.186	163.163	235.235	127.127	135.137	132.132	214.214	137.137	188.192		M	
HS'465	13	140.140	203.207	172.186	163.163	229.235	109.127	135.137	132.132	206.206	137.137	192.192	157.157	M	
Marina (F10)	13	140.156	203.207	172.172	157.163	229.235	109.127	137.137	136.136	206.206	135.135	188.188	157.157	F	Gen1.54
RAM'011	13	140.140	203.203	172.184	163.163	229.235	127.127	137.139	132.136	206.212	135.137	192.192	157.157	F	Gen1.58
RAM'024	13	156.156	203.207	172.172	157.163	229.235	127.129	135.139	132.136	206.214	135.135	188.192	157.157	M	
RAM'0529	12	140.140	207.207	186.186	163.163	229.229	109.129	135.137	132.132	206.214	137.137	192.192		M	
ram'0587	13	140.156	203.207	172.186	163.163	229.235	127.127	137.137	132.132	206.212	135.137	192.192	157.157	M	
RAM'072	13	140.156	203.207	172.184	163.163	229.235	127.129	137.139	132.136	206.212	135.137	192.192	157.157	F	Gen1.43
Reginella (F01)	13	140.156	203.207	172.186	157.163	229.229	127.129	135.137	132.132	206.214	135.137	192.192	157.157	F	Gen1.25
RT'0348	12	156.156	207.207	172.186	163.163	229.235	127.127	139.139	132.132	206.214	135.137	192.192		F	
RT'0429	13	140.156	207.207	172.186	157.163	229.229	109.127	137.137	132.132	206.212	135.137	188.192	157.157	M	
RT'0597	13	140.140	203.207	172.186	163.163	229.235	127.127	135.137	132.136	206.206	137.137	192.192	157.157	M	
RT'109	13	140.140	203.203	172.172	163.163	229.235	127.127	135.137	132.132	206.214	137.137	192.192	157.157	F	
RT'148	12	140.140	203.203	172.186	157.163	235.235	127.127	139.139	132.136	214.214	137.137	192.192		M	
RT'187	13	156.156	207.207	172.172	157.163	229.229	127.127	137.139	132.136	206.206	135.135	188.192	157.157	F	Gen1.37
Sebastiana (F08)	13	140.140	203.207	184.186	157.163	229.235	109.127	135.137	132.136	206.206	137.137	188.192	157.157	F	Gen1.73
Stella (F02)	13	156.156	203.207	172.172	157.163	229.229	109.109	137.137	132.132	206.212	135.135	188.192	145.157	F	Gen1.56
Tommaso (M09)	13	140.156	203.207	172.186	163.163	229.229	109.109	137.137	132.132	206.212	135.137	188.192	157.157	M	Gen1.71 (1MM)
Tranquilla (F13)	13	156.156	203.207	172.186	163.163	235.235	127.129	135.139	132.136	214.214	137.137	192.192	157.157	F	Gen1.31
Ura (F07)	13	140.156	203.207	172.186	157.163	229.235	109.127	137.139	132.136	212.212	135.137	188.192	145.157	F	Gen1.23

Appendix B
continued

Genotype	# loci	G10B	G10C	G1D	G10L	MU59	REN144 A06	CXX20	MU50	MU51	MU05	MU11	G10P	Sex	Matches with ISPRA dataset
Valerie (F03)	13	140.156	203.203	172.186	157.157	229.229	109.127	139.139	132.132	212.212	137.137	188.192	157.157	F	Gen1.44
Vittorio (M13)	13	140.156	203.207	172.186	157.157	229.235	109.127	137.139	132.132	206.212	137.137	188.192	157.157	M	Gen1.66
ORSNEC0302	13	156.156	203.207	172.172	157.163	229.229	127.129	137.137	132.132	206.212	135.135	188.192	145.157	F	Gen1.13
ORSNEC0114	13	140.140	207.207	172.186	163.163	229.235	109.129	137.137	132.132	206.206	135.137	188.192	157.157	F	

Appendix C

Encounter histories for 43 Apennine brown bears sampled in the 2014 survey in the PNALM ecosystem (June – September) and used for CR modelling to estimate population size. The bears live-trapped during a previous study are identified with their code (e.g., F05), whereas all other bears are coded with the identification code of the first hair-sample through which they have been identified. For each individual (row), columns III-XXII report capture (1) or no capture (0) in each session by sampling method (*Table continues in the next page*).

Bear	Sex	Hair-snagging					Buckthorn sampling				Rub-tree sampling										ACC	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		20
89_ACC_01	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
89_ACC_03a	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
89_ACC_04	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Acc'079	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Atessa (F05)	F	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	1
Cicerone (M12)	M	1	0	0	1	0	0	1	0	0	0	1	1	1	0	0	1	1	1	1	0	0
Forchetta	F	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1
Gemma (FP01)	F	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
HS'028	F	0	0	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
HS'0853	F	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS'0956	F	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS'0980	M	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS'1008	F	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
HS'1139	M	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS'1192	M	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
HS'1293	M	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
HS'330	F	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
HS'338	M	1	1	1	0	1	0	0	0	0	1	0	1	1	1	0	0	1	0	1	1	1
HS'343	F	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
HS'349	M	1	0	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	0	1	1	1
HS'355	M	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
HS'374	M	1	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	1
HS'451	M	1	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	0	0	0	0
HS'465	M	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	1	1	1
Marina (F10)	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1
RAM'011	F	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
RAM'024	M	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
RAM'0529	M	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
ram'0587	M	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
RAM'072	F	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Reginella (F01)	F	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT'0348	F	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
RT'0429	M	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
RT'0597	M	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
RT'109	F	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
RT'187	F	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0

Appendix C
continued

Bear	Sex	Hair-snagging					Buckthorn sampling					Rub-tree sampling										Acc
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Sebastiana (F08)	F	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	
Stella (F02)	F	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	
Tommaso (M09)	M	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	
Tranquilla (F13)	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
Ura (F07)	F	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
Valerie (F03)	F	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	
Vittorio (M13)	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	