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## Evidence of a large carnivore population recovery: Counting bears in

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Abstract

Keywords: brown bear, Greece, Jolly-Seber capture-mark-recapture modelling framework, nonivasive genetic sampling, population monitoring, power pole hair traps, Ursus arctos

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## Introduction

The brown bear (Ursus arctos) is a charismatic key species of the biodiversity of the Holarctic region (Servheen, Herrero \& Peyton, 1999), capable of attracting attention and resources to conservation efforts through its function as an iconic flagship species (Simberloff, 1999). Once, extirpated and threatened by extinction throughout large parts of Europe (Zedrosser et al., 2001), bears have made a remarkable recovery, with populations increasing and expanding in several countries (Deinet et al., 2013). The benefits for nature conservation notwithstanding, such recoveries create the potential of increased wildlife mortality and human - wildlife conflicts (Gardner et al., 2010). In this context, informed, science-based conservation and management decisions are urgently required to safeguard the recovery and survival of small brown bear populations, which has been identified as an important European conservation priority (Habitat Directive 92/43/CEE). Since conservation priorities often arise in relation to bear numbers, reliable population assessments are fundamental in conservation and management planning (Mowat \& Strobeck, 2000, Lorenzini et al., 2004, Kendall et al., 2008).

Brown bears in Greece reach their southern-most distribution in Europe and are therefore an important aspect of European biodiversity. They belong to the Dinaric-Pindos (DP) bear population, which has been identified as one of the largest and most important on the European continent (Zedrosser et al., 2001); the DP bear population appears to be stable, numbering more than 3,000 individuals (Deinet et al., 2013, Kaczensky et al., 2013). In Greece, brown bears are considered to be endangered (Mertzanis, Giannakopoulos \& Pylidis, 2009).

In recent years the increase of extra-limital appearances of bears throughout the country (Karamanlidis, Krambokoukis \& Kantiros, 2008), and the increase in negative human - bear interactions (i.e., mainly damages by bears to human property) (Karamanlidis et al., 2011) and bear - vehicle collisions (Karamanlidis et al., 2012) suggest that the brown bear population in Greece might be recovering. The arising conservation issues of this population expansion and growth make the development and implementation of an effective Action Plan for the species an immediate conservation priority (Mertzanis et al., 2009).

Despite public interest and costly management programs, no rigorous and reliable estimates of abundance and density of brown bears in Greece exist. Because of their crepuscular and nocturnal activity patterns, large home range size, shyness, low density and the rugged and forested terrain they inhabit, counting brown bears by traditional field methods (e.g., direct observations, extrapolations of counts of mother with cubs of the year) is expensive or sometimes even unfeasible (Mace et al., 1994). Traditional field methods are viewed as less rigorous than more efficient current capture-mark-recapture (CMR) and DNA-based methods (Garshelis, 2006). The size given therefore for the brown bear population in Greece [i.e., 190 - 260 individuals (Mertzanis et al., 2009)], which is presumably a combination of expert knowledge and observation counts, should be considered an educated guess that might not reflect actual population processes (Nichols \& Williams, 2006). The current lack of reliable data for the brown bear population in Greece sharply contradicts with the need to develop a sound, effective and outreaching management and conservation strategy in the country (Ciucci \& Boitani, 2008).

Genetic methods play a pivotal role in bear conservation and cannot be neglected when developing effective conservation strategies and planning the long-term survival of threatened bear populations (Swenson, Taberlet \& Bellemain, 2011). One of the most attractive applications of molecular methods, commonly used now by management agencies (McCall et al., 2013), is the possibility of estimating the (minimum) number of free-ranging individuals (Schwartz, Tallmon \& Luikart, 1998; Kohn et al., 1999). Coupled with noninvasive genetic sampling (NGS), which is ideal for studying small, endangered populations as it precludes the unnecessary capture of individuals, and CMR methods, genetic study methods have been successfully deployed to obtain bear abundance and density estimates for large (Mowat \& Strobeck, 2000, Boersen, Clark \& King, 2003, McCall et al., 2013) and small study areas with irregular sampling designs and small population sizes [<100 individuals (Gardner, Royle \& Wegan, 2009, Obbard, Howe \& Kyle, 2010, Gervasi et al., 2012, Latham et al., 2012)].

The goal of our study was to apply noninvasive genetic sampling as part of a capture-mark-recapture (CMR) study design for estimating bear abundance and density in Greece.

## Methods

## Study area

Brown bears in Greece occur in two disjunct populations in the western and eastern part of the country (Figure 1A); the population in the western part of the country is stable or locally increasing and is estimated to number approximately $190-230$ individuals (Mertzanis et al., 2009). The study was carried out in the
western part of the brown bear range in Greece, at the approximately 250 kmlong Greek part of the Pindos mountain range, extending from the Albanian and the Former Yugoslav Republic (FYR) of Macedonian - Greek border in the northwest of the country to the region of Central Greece (Figure 1B). The study area forms a mosaic of elevations and habitats, from deep canyons and low altitudes to steep and high mountains. Elevations range from a couple of hundred meters above sea up to the peak of Mount Smolikas at 2,637 m above sea level. This wide range of elevations results in two major forest zones: A higher elevation conifer zone, with tree species such as the endemic Greek fir (Abies cephalonica), the European black pine (Pinus nigra) and the Bosnian pine (Pinus heldreichii) and a lower elevation mixed broadleaf forest, which is dominated by oak (Quercus sp.), beech (Fagus sp.) and fir (Abies sp.). The local fauna includes other large and medium-sized carnivores, such as the grey wolf (Canis lupus) and the golden jackal (Canis aureus), and intensity of human activity ranges from areas with low human presence to major cities with populations > 50,000.

## Sampling methods

Genetic sampling was based on the natural marking and rubbing behavior of bears (Green \& Mattson, 2003) on wooden poles of the telephone and electricity network (hereafter power poles) in Greece (Karamanlidis et al., 2007) and therefore we did not use any attractant to lure bears to the sampling stations. In the preparatory phase of the study (2002-2006) we inspected and evaluated the suitability of power poles as non-invasive genetic sampling stations according to predefined criteria that considered among others marking and rubbing frequency
and intensity, type of habitat, vegetation density and intensity of human activity (Karamanlidis, 2008); then we conducted a pilot study that confirmed the suitability of power poles for the non-invasive genetic monitoring of brown bears (Karamanlidis et al., 2010).

For the purposes of this study and following the inspection and evaluation of 4147 power poles in the study area we selected 171 and placed barbed-wire hair traps (Kendall \& McKelvey, 2008) on them, thus creating four different sampling areas (i.e., Vitsi - Varnoundas, and Northern, Central and Southern Pindos) (Figure 1B, Table 1). We selected location and size of the sampling areas so as to cover the maximum of the core range of the species in the Pindos mountain range; sampling areas were separated either by distance or/and geomorphological features (e.g. extensive plains and valleys) and human infrastructure (e.g. major highways). We calculated the size of the sampling areas by drawing a buffer zone of 5.86 km around the Minimum Convex Polygon (MCP) defined by the outermost sampling stations in each study area; 5.86 km was the mean distance between all the individual bear recaptures recorded during the study. We carried out systematic sampling efforts in all four sampling areas from October 2007 to October 2010, when poles were inspected and hair samples were collected monthly.

## Figure 1

Figure 1 A) Map of southeastern Europe. The shaded areas indicate the approximate geographic range of brown bears in the region. B) Geophysical map of Greece indicating the location of the study area. C) Map of Greece indicating the location of four sampling areas for the non-invasive collection of brown bear collection of genetic samples from brown bears in Greece (2007-2010).

| Sampling area | Location | Sampling area size $\left(\mathbf{k m}^{2}\right)$ | Sampling stations $(\boldsymbol{N})$ |
| :--- | :---: | :---: | :---: |
| Vitsi - Varnoundas | N: 40.6935, | 1270.39 | 40 |
|  | E: 21.3192 |  |  |
| Northern Pindos | N: 40.3707, | 1066.22 | 45 |
|  | E: 21.0907 |  | 47 |
| Central Pindos | N: 39.9197, | 999.72 | 47 |
|  | E: 21.4054 |  | 39 |
| Southern Pindos | N: 39.6305, | 954.33 |  |
|  | E: 21.3711 |  | $\mathbf{1 7 1}$ |
| Total |  | 4290.66 |  |

genetic samples (2007-2010). D) The inset photograph pictures an adult female brown bear with her cubs rubbing and marking a power pole.

Table 1 Descriptive statistics of four sampling areas for the non-invasive

## Genetic Methods

Each tuft of hairs on a set of barbs was considered a sample; hair samples were collected without contact to human skin, were placed in uniquely numbered paper envelopes and then stored at room temperature in zip-lock bags with silica gel (Roon, Waits \& Kendall, 2003) until being analyzed by Wildlife Genetics International (Nelson, British Columbia, Canada), a laboratory that specializes in non-invasively obtained samples that often have low quantities of DNA. DNA was extracted using the DNeasy Blood \& Tissue kits (QIAGEN, Hilden, Germany), following the manufacturer's instructions. All extractions took place in a facility in which amplified DNA had never been handled before.

To determine individual identity each sample was genotyped at the microsatellite loci G1D, G10J, G10L (Paetkau \& Strobeck, 1994, Paetkau, Shields \& Strobeck, 1998), G10C, G10P (Paetkau et al., 1995), MU51 and MU59
(Taberlet et al., 1997). Gender identification was established through the analysis of the amelogenin gene (Ennis \& Gallagher, 1994). Up to 10 additional loci were analyzed for $\geq 1$ sample from each individual to enable more detailed population genetic analyses. These extended genotypes were used to confirm differences between individuals with similar 7-locus genotypes.

Thermal cycling was performed using a MJ Research PTC100 thermocycler with 96 well 'Gold’ blocks (MJ Research Inc., St. Bruno, Quebec, Canada). Polymerase chain reaction (PCR) buffers and conditions were used according to Paetkau et al. (1998), except that markers were not co-amplified, because coamplification may reduce the success rates for hair samples (D. Paetkau, Wildlife Genetics International, personal communication). Two mM MgCl2 was used for all markers except G10J (1.8 mM). An automated sequencer (ABI 310) was used, and genotypes were determined using ABI Genescan and Genotyper version 2.1 software (Perkin Elmer-Applied Biosystems, Foster City, California, USA). The sizing of the PCR products was performed using capillary electrophoresis.

Mixed samples (samples with hair from $>1$ bear) were reliably identified by evidence of $\geq 3$ alleles at $\geq 1$ locus (Roon et al., 2005). To minimize genotyping errors in the final data set, low-quality and putatively mixed samples were excluded from further analyses (Paetkau 2003). Genotypes were replicated for all 1 ) individuals identified in one sample, 2) pairs of individuals that differed at only 1 or 2 loci (1- and 2-mismatch pairs) and 3) pairs of individuals that differed at 3 loci when $\geq 1$ locus was consistent with allelic dropout. Test for allelic dropout, presence of null alleles, and scoring errors caused by stutter peaks were
performed with Micro-Checker version 2.2.3 (van Oosterhout, Hutchinson \& Wills, 2004).

We used the observed number of alleles $(A)$ and expected heterozygosity $\left(H_{e}\right)$ to express genetic variation in our population. We calculated the probability of identity ( $P_{I D}$ ) and of siblings ( $P_{\text {SIB }}$ ) to describe the power of our markers to identify individuals (Waits, Luikart \& Taberlet, 2001) using the software GIMLET version 1.3.2 (Valière 2002). To allow for the possibility of mismatches caused by genotyping error, we also looked for the pairs of genotypes that were matched at all but 1, 2, and 3 loci (1-MM, 2-MM, and 3-MM pairs) using the program GenAIEx 6 (Peakall and Smouse 2006).

## Estimating Abundance and Density

To estimate abundance, we used the Jolly-Seber (JS) CMR modeling framework that allows accounting for detectability less than one and dealing with open populations, i.e., systems in which births and deaths occur (Jolly, 1965, Seber, 1965). JS models provide estimates of abundance, probabilities of survival, detection and recruitment [sensu (Schwarz \& Arnason, 1996) i.e., the probability that an animal from the hypothetical super-population would enter the population on a particular time interval]. We analyzed each of the four regions separately. We considered time and sex effects as possibly explaining environmental and individual variation in these demographic rates, entering in an additive or interactive way in the model, which led to a total of 125 candidate models. To select among these models, we used the Akaike's Information Criterion corrected for small sample size (AICc) and considered the model with the lowest AICc value as being the model that best balanced bias and precision (Burnham \&

Anderson, 2002). In addition, we used changes in AICc values ( $\triangle \mathrm{AICc}$ ) to compare model support with reference to the model best supported by the data. Annual abundance estimates were obtained by model averaging in which each model contributed to the final estimate according to its AICc weight (Burnham \& Anderson, 2002). The analyses were performed using POPAN (Arnason \& Schwarz, 1995) available in MARK (White \& Burnham, 1999) that was called from R (R Development Core Team, 2011) with package RMark (Laake \& Rexstad, 2008).

We tested the validity of important assumptions underlying the safe use of the JS model like the presence of transient individuals or an effect of trapping using standard goodness-of-fit tests (Pradel, Gimenez \& Lebreton, 2005) as implemented in program U-CARE (Choquet et al., 2009).

Although we considered a potential effect of sex on the detection probability, other sources of individual heterogeneity might go undetected in the present analysis, which could lead to severe bias in abundance estimates (e.g., (Cubaynes et al., 2010b). This might be for example due to individuals being more active than others at the proximity of power poles, hence making them more capturable. To assess whether some individual heterogeneity was present besides gender effects, we compared for each of the four study areas the model best supported by the data to its counterpart, incorporating heterogeneity in the detection probability. To do so, we considered finite-mixture JS models (Pledger, Pollock \& Norris, 2010) that extend standard JS models by assuming that the animals come from different classes of detection, although we do not know which class each individual is from. Heterogeneous JS models were fitted using the R
package 'hetage' that is available from http://homepages.ecs.vuw.ac.nz/~shirley/. To compare homogeneous and heterogeneous models, AIC could not be used because of the violation of regularity conditions (e.g. (Gimenez \& Choquet, 2010) in a CMR context), and we resorted to a likelihood ratio test that was distributed under the null hypothesis of homogeneous detection probabilities as a 50:50 mixture of chi-square distributions with 0 and 1 degrees of freedom (Self \& Liang, 1987). Note that, to get the P-value of this test, using the mixture corresponds to halving the P -value from using the standard chi-square distribution with 1 degree of freedom.

We calculated bear density using the sampled area size of each sampling network respectively (i.e., Vitsi - Varnounds: $1270.39 \mathrm{~km}^{2}$; Northern Pindos: $1066.22 \mathrm{~km}^{2}$; Central Pindos: $999.72 \mathrm{~km}^{2}$; Southern Pindos: $954.33 \mathrm{~km}^{2}$, Table 1). Less than $3 \%$ of the study areas were not suitable habitat for bears (e.g., lakes, rivers and human settlements); therefore we retained these areas in the sampled area and density calculations.

## Results

## Sampling Effort

From 2007-2010, we conducted 4422 inspections to the 171 sampling sites and collected 860 hair samples (Table 2). We collected bear hair at $92.25 \%$ of all hair traps (i.e., Vitsi - Varnoundas: 95\%; Northern Pindos: 100\%; Central Pindos: 95\%; Southern Pindos: $79 \%$ ) during $25.75 \%$ of all power pole visits (i.e., Vitsi Varnoundas: 30\%; Northern Pindos: 34\%; Central Pindos: 26\%; Southern Pindos: 13\%).

1 Of the 860 samples sent to the laboratory, we excluded 250 (29\%) from the 2 analysis due to insufficient genetic material (i.e., hair without follicles). Of the 610 3 samples that we attempted to analyze, 11 (1.8\%) appeared to contain DNA from $4>1$ individual, and 154 samples (25\%) failed the DNA extraction process. From 5 the remaining 445 samples (64\% of total collected) 211 (61F, 151M) individuals 6 were identified and 102 individuals were recaptured (Table 2). From the 211 7 individuals identified, 206 individuals (98\%) produced complete 7-locus genotypes plus gender assignment.

9 Table 2. Descriptive statistics of efforts at four sampling areas in Greece (20072010) for the non-invasive collection of brown bear hair samples

| Sampling area | Visits | Bear <br> presence | Hair <br> samples <br> collected | Females <br> identified | Males <br> identified | Total $\boldsymbol{N}$ <br> identified | $\boldsymbol{N}$ of <br> animals <br> recaptured |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Vitsi | -1115 | 358 | 215 | 13 | 39 | 52 | 28 |
| Varnoundas |  |  |  |  |  |  |  |
| Northern Pindos | 1146 | 421 | 295 | 26 | 51 | 77 | 29 |
| Central Pindos | 1220 | 351 | 249 | 19 | 42 | 61 | 33 |
| Southern Pindos | 941 | 132 | 101 | 3 | 18 | 21 | 12 |
| Total | $\mathbf{4 4 2 2}$ | $\mathbf{1 2 6 2}$ | $\mathbf{8 6 0}$ | $\mathbf{6 1}$ | $\mathbf{1 5 0}$ | $\mathbf{2 1 1}$ | $\mathbf{1 0 2}$ |

11 Mean observed heterozygocity across the 7 markers used to identify individuals was 0.70 (Table 3). The probability that 2 randomly drawn, unrelated individuals would share the same genotype $\left(P_{I D}\right)$ was 0.0000005 , and the probability that full siblings would have identical genotypes $\left(P_{\text {SIB }}\right)$ was 0.002 (Table 3). Based on the observed distribution of genotype similarity for the 7 loci used for individual identifications, we predicted that no pair of matching genotypes could exist within our dataset. Of the 211 individuals present in our

1 analysis, $91 \%$ had $\geq 10$-locus genotypes and, when all available loci were 2 considered, all individual bears differed at $\geq 3$ loci.

Table 3. Variability of microsatellite markers used to determine individual identity 4 of brown bears in Greece, 2007-2010.

| Marker | $\boldsymbol{H}_{E}{ }^{a}$ | $\boldsymbol{H}_{0}{ }^{a}$ | $\boldsymbol{A}^{a}$ | $\boldsymbol{P}_{I D}{ }^{a}$ | $\boldsymbol{P}_{\text {SIB }}{ }^{a}$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| G1D | 0.78 | 0.80 | 6 | 0.08 | 0.37 |
| MU59 | 0.78 | 0.76 | 8 | 0.07 | 0.38 |
| G10C | 0.76 | 0.76 | 6 | 0.09 | 0.39 |
| G10P | 0.75 | 0.67 | 9 | 0.10 | 0.40 |
| G10J | 0.70 | 0.77 | 6 | 0.14 | 0.43 |
| G10L | 0.57 | 0.55 | 5 | 0.23 | 0.52 |
| MU51 | 0.55 | 0.57 | 5 | 0.24 | 0.53 |
| $\bar{X}$ | 0.70 | 0.70 | 6.42 |  |  |
| Overall probability of identity |  |  |  | $5.299 \mathrm{e}-07$ | 0.002 |

${ }^{\text {a }} H_{E}=$ expected heterozygosity; $H_{O}=$ observed heterozygosity; $A$ number of alleles; $P_{I D}=$ probability of identity; $P_{S I B}=$ probability of identity among siblings

## Abundance and density estimation

The standard assumptions of the JS model were valid as showed by the goodness-of-fit tests that were non-significant for all regions $\left(\chi_{3}^{2}=2.45, \mathrm{p}\right.$-value $=$ 0.49 for Vitsi - Varnoundas; $\chi_{4}^{2}=3.58, p$-value $=0.47$ for Northern Pindos; $\chi_{7}^{2}=$ $0.47, p$-value $=0.99$ for Central Pindos; $\chi_{2}^{2}=0.71, p$-value $=0.70$ for Southern Pindos). Model selection showed contrasted effects between regions (Table 4). Survival was constant in Vitsi - Varnoundas and Southern Pindos and sexspecific in Central and Northern Pindos with the addition of time in the latter. Recruitment was constant in all regions but Northern Pindos in which it was sex
and time dependent on an additive scale. Detection was constant in all regions but Vitsi - Varnoundas in which it was sex-specific. There was substantial uncertainty regarding the best model to select for all regions, with several models within the range $\triangle \mathrm{AICc}<2$, which motivated the use of model-averaged abundance estimates (see below). We did not detect extra heterogeneity in the detection probability $\left(\chi_{1}^{2}=2.5, p\right.$-value $=0.06$ for Vitsi - Varrnoundas; $\chi_{4}^{2}=2.6$, $p$-value $=0.31$ for Northern Pindos; $\chi_{7}^{2}=8.5, p$-value $=0.15$ for Central Pindos; $\chi_{1}^{2}=0.6, p$-value $=0.22$ for Southern Pindos).

Table 4. Model selection results from CMR analysis of the brown bear populations in Greece using the Jolly-Seber modelling framework. For each of the four regions (i.e., Vitsi - Varnoundas, Northern, Central and Southern Pindos), the Akaike's Information Criterion value corrected for small sample sizes (AICc), the difference in AICc value between the th model and the model with the lowest AICc value ( $\triangle$ AICc). Constant (.), sex (s) and time (t) effects were considered on survival $(\varphi)$, detection (p) and entry (b) probabilities either as main effect, in an additive (+) or in interactive (x) fashion. Note that, for each of the four regions, only the 10 top ranked models are displayed although 125 models were originally fitted to the data.

| Region | Model | k | AICc | $\boldsymbol{\Delta A I C c}$ |
| :--- | :--- | :--- | :--- | :--- |
|  | $\varphi() .\mathrm{p}(\mathrm{s}) \mathrm{b}()$. | 5 | 95.17 | 0.00 |
|  | $\varphi(\mathrm{~s}+\mathrm{t}) \mathrm{p}() .\mathrm{b}(\mathrm{s})$ | 7 | 96.49 | 1.34 |
| Vitsi- | $\varphi(\mathrm{s}) \mathrm{p}() .\mathrm{b}(\mathrm{s})$ | 6 | 97.51 | 2.36 |
| Varnoundas | $\varphi(\mathrm{t}) \mathrm{p}(\mathrm{s}) \mathrm{b}()$. | 6 | 97.51 | 2.37 |
|  | $\varphi() .\mathrm{p}(\mathrm{s}) \mathrm{b}(\mathrm{s})$ | 6 | 97.54 | 2.39 |
|  | $\varphi() .\mathrm{p}(\mathrm{s}) \mathrm{b}(\mathrm{t})$ | 6 | 97.57 | 2.42 |
|  | $\varphi(\mathrm{~s}) \mathrm{p}(\mathrm{s}) \mathrm{b}()$. | 6 | 97.58 | 2.43 |


|  | $\varphi(\mathrm{s}) \mathrm{p}() .\mathrm{b}($. | 5 | 97.91 | 2.76 |
| :---: | :---: | :---: | :---: | :---: |
|  | $\varphi(s+t) p() b.($. | 6 | 98.26 | 3.12 |
|  | $\varphi() p.() b.(s)$ | 5 | 98.37 | 3.22 |
| Northern Pindos | $\varphi(s+t) p() b.(s+t)$ | 7 | 126.03 | 0.00 |
|  | $\varphi(s) p() b.(s+t)$ | 8 | 126.19 | 0.16 |
|  | $\varphi(s+t) p(s) b(s+t)$ | 8 | 127.97 | 1.94 |
|  | $\varphi(\mathrm{s}) \mathrm{p}(\mathrm{t}) \mathrm{b}(\mathrm{s}+\mathrm{t})$ | 11 | 128.02 | 2.00 |
|  | $\varphi(s) p(s) b(s+t)$ | 10 | 128.47 | 2.48 |
|  | $\varphi(s+t) p() b.(t)$ | 11 | 128.67 | 2.64 |
|  | $\varphi(\mathrm{s}) \mathrm{p}() .\mathrm{b}(\mathrm{t})$ | 8 | 128.82 | 2.79 |
|  | $\varphi(s) p(t) b(t)$ | 9 | 129.09 | 3.06 |
|  | $\varphi(\mathrm{s}) \mathrm{p}(\mathrm{t}) \mathrm{b}(\mathrm{s})$ | 9 | 129.41 | 3.38 |
|  | $\varphi(s+t) p(t) b(s+t)$ | 9 | 129.95 | 3.92 |
| Central Pindos | $\varphi(\mathrm{s}) \mathrm{p}() .\mathrm{b}($. | 8 | 143.03 | 0.00 |
|  | $\varphi(\mathrm{s}) \mathrm{p}() .\mathrm{b}(\mathrm{t})$ | 8 | 143.64 | 0.61 |
|  | $\varphi() p.(s) b($. | 9 | 143.77 | 0.74 |
|  | $\varphi(\mathrm{s}+\mathrm{t}) \mathrm{p}() .\mathrm{b}($. | 7 | 144.56 | 1.54 |
|  | $\varphi() p.() b.($. | 10 | 144.73 | 1.71 |
|  | $\varphi(\mathrm{s}) \mathrm{p}() .\mathrm{b}(\mathrm{s})$ | 9 | 144.75 | 1.72 |
|  | $\varphi(\mathrm{s}+\mathrm{t}) \mathrm{p}() .\mathrm{b}(\mathrm{t})$ | 7 | 144.76 | 1.73 |
|  | $\varphi(\mathrm{s}) \mathrm{p}() .\mathrm{b}(\mathrm{s}+\mathrm{t})$ | 10 | 144.82 | 1.79 |
|  | $\varphi() p.(s) b(t)$ | 8 | 144.82 | 1.79 |
|  | $\varphi(\mathrm{s}) \mathrm{p}(\mathrm{s}) \mathrm{b}($. | 11 | 144.83 | 1.80 |
| Southern Pindos | $\varphi() p.() b.($. | 4 | 55.05 | 0.00 |
|  | $\varphi(\mathrm{s}) \mathrm{p}() .\mathrm{b}($. | 5 | 55.14 | 0.09 |
|  | $\varphi() p.(s) b($. | 6 | 57.84 | 2.78 |
|  | $\varphi(\mathrm{t}) \mathrm{p}() .\mathrm{b}($. | 7 | 57.96 | 2.91 |
|  | $\varphi() p.() b.(s)$ | 5 | 57.98 | 2.93 |
|  | $\varphi(s) p(s) b($. | 6 | 58.00 | 2.95 |
|  | $\varphi() .\mathrm{p}() .\mathrm{b}(\mathrm{t})$ | 6 | 58.04 | 2.99 |
|  | $\varphi(s+t) p() b.($. | 5 | 58.28 | 3.23 |
|  | $\varphi(\mathrm{s}) \mathrm{p}() .\mathrm{b}(\mathrm{s})$ | 8 | 58.39 | 3.34 |
|  | $\varphi(\mathrm{s}) \mathrm{p}() .\mathrm{b}(\mathrm{t})$ | 6 | 58.41 | 3.36 |

1 The most abundant region was Vitsi - Varnoundas with 68 individuals on
2 average over the study period, followed by Northern Pindos with 53 individuals,
3 Central Pindos with 51 individuals and Southern Pindos with 10 individuals 4 (Table 5). Table 5. Abundance estimates from CMR analysis of the brown bear populations in Greece using the Jolly-Seber modeling framework. For each of the four regions (i.e., Vitsi - Varnoundas and Northern, Central and Southern Pindos), the time and sex-specific population size estimate, as well as the lower (LCL) and upper (UCL) confidence limits of the $95 \%$ confidence interval obtained from model averaging are given.

| Region | Sex | Estimate |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Vitsi Varnoundas |  |  | 2007-8 | 2008-9 | 2009-10 |
|  |  | value | 18.7 | 22.4 | 21.4 |
|  | female | LCL | 4.9 | 7.1 | 5.2 |
|  |  | UCL | 71.1 | 70.8 | 88.3 |
|  |  | value | 43.0 | 47.4 | 50.8 |
|  | male | LCL | 12.4 | 15.1 | 15.6 |
|  |  | UCL | 148.7 | 148.6 | 165.2 |
|  |  | value | 61.7 | 69.8 | 72.2 |
|  | pooled | LCL | 19.7 | 24.7 | 23.6 |
|  |  | UCL | 193.0 | 197.6 | 220.6 |
| Northern Pindos |  | value | 16.9 | 19.7 | 4.0 |
|  | female | LCL | 8.7 | 10.1 | 0.7 |
|  |  | UCL | 32.8 | 38.5 | 24.5 |
|  |  | value | 44.9 | 46.5 | 27.7 |
|  | male | LCL | 20.6 | 26.7 | 9.1 |
|  |  | UCL | 97.7 | 81.3 | 84.6 |
|  | pooled | value | 61.8 | 66.3 | 31.8 |
|  | pooled | LCL | 31.1 | 38.7 | 9.9 |



## 1

 7 remarkable consistency of our estimates between years (especially the years 82008 and 2009) lends credibility to our results.9 There are several assumptions that need to be checked to use the Jolly-Seber

## Discussion

We present the first rigorous estimate of brown bear abundance and density in the western range of brown bears in Greece. By using genetic data from hair samples collected from poles of the electricity and telephone network, an approach that has not been implemented in this scale before, we obtained precise population estimates despite fairly low capture and recapture rates. The model and obtain reliable abundance estimates. First, we assumed that there
was no immigration or emigration (i.e., closure assumption) in the four regions we studied. This was not entirely true as several individuals moved from region $X X$ to region $X X$ during the study period. A modelling option would have been to use multisite JS models (e.g., (Lebreton et al., 2009) instead of considering the region as a factor, hence allowing the estimation of movement between regions. However, with so few individuals actually making the transitions between regions, fitting multisite JS models would be numerically unstable with movement parameters estimated on the boundary (Gimenez et al., 2005) and identifiability issues (Gimenez, Choquet \& Lebreton, 2003). Add here a sentence on how we should interpret our abundance estimates consequently. A second assumption underlying the application of JS models is that all animals, marked or unmarked, should have the same capturability (i.e., homogeneous capturability assumption). Here, there was no reason to believe that the use of poles to "capture" individuals would distinguish between marked and unmarked individuals. Third, the duration of the sampling period should be short in comparison to the time between sampling occasions. In our study, most of the observations were made over eight months, which was considered a short enough with regard to the yearly time interval we used between sampling periods as bear survival was high (Hargrove \& Borland, 1994). Fourth, and probably the most crucial assumption, detection probabilities were assumed homogeneous between individuals, heterogeneity if present and ignored being source of strong bias in the abundance estimates (Cubaynes et al., 2010a). We found a sex effect for the Vitsi - Varnoundas population, males being more detectable than females (0.18 [SE=0.12] for females and 0.38 [SE=0.08] for males), and this difference was accounted for when calculating abundance estimates. We also tested for extra individual
heterogeneity not explained by differences between males and females, but did not find any, which suggest that the risk of bias in abundance estimates was avoided. Last but not least, it is usually assumed that individuals retain their mark throughout the study and that these marks are read correctly. When interpreted in the context of DNA marking, these two assumptions refer to data quality and the possibility of genotyping error. Last but not least, issue of the coverage of the poles.

## Management implications

Importance of estimating abundance for conservation and management. Originality of the poles as non-invasive monitoring method. Very efficient when combined with capture-recapture methods that account for detection $<1$ and demography (mortality / birth).

## Conclusions

## Acknowledgements

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