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

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1 **Abstract**

2

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

6

1 **Introduction**

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

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

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

9 Despite public interest and costly management programs, no rigorous and
10 reliable estimates of abundance and density of brown bears in Greece exist.
11 Because of their crepuscular and nocturnal activity patterns, large home range
12 size, shyness, low density and the rugged and forested terrain they inhabit,
13 counting brown bears by traditional field methods (e.g., direct observations,
14 extrapolations of counts of mother with cubs of the year) is expensive or
15 sometimes even unfeasible (Mace *et al.*, 1994). Traditional field methods are
16 viewed as less rigorous than more efficient current capture-mark–recapture
17 (CMR) and DNA-based methods (Garshelis, 2006). The size given therefore for
18 the brown bear population in Greece [i.e., 190 – 260 individuals (Mertzanis *et al.*,
19 2009)], which is presumably a combination of expert knowledge and observation
20 counts, should be considered an educated guess that might not reflect actual
21 population processes (Nichols & Williams, 2006). The current lack of reliable data
22 for the brown bear population in Greece sharply contradicts with the need to
23 develop a sound, effective and outreaching management and conservation
24 strategy in the country (Ciucci & Boitani, 2008).

1 Genetic methods play a pivotal role in bear conservation and cannot be
2 neglected when developing effective conservation strategies and planning the
3 long-term survival of threatened bear populations (Swenson, Taberlet &
4 Bellemain, 2011). One of the most attractive applications of molecular methods,
5 commonly used now by management agencies (McCall *et al.*, 2013), is the
6 possibility of estimating the (minimum) number of free-ranging individuals
7 (Schwartz, Tallmon & Luikart, 1998; Kohn *et al.*, 1999). Coupled with noninvasive
8 genetic sampling (NGS), which is ideal for studying small, endangered
9 populations as it precludes the unnecessary capture of individuals, and CMR
10 methods, genetic study methods have been successfully deployed to obtain bear
11 abundance and density estimates for large (Mowat & Strobeck, 2000, Boersen,
12 Clark & King, 2003, McCall *et al.*, 2013) and small study areas with irregular
13 sampling designs and small population sizes [<100 individuals (Gardner, Royle &
14 Wegan, 2009, Obbard, Howe & Kyle, 2010, Gervasi *et al.*, 2012, Latham *et al.*,
15 2012)].

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

19 **Methods**

20 **Study area**

21 Brown bears in Greece occur in two disjunct populations in the western and
22 eastern part of the country (Figure 1A); the population in the western part of the
23 country is stable or locally increasing and is estimated to number approximately
24 190 – 230 individuals (Mertzanis *et al.*, 2009). The study was carried out in the

1 western part of the brown bear range in Greece, at the approximately 250 km-
2 long Greek part of the Pindos mountain range, extending from the Albanian and
3 the Former Yugoslav Republic (FYR) of Macedonian - Greek border in the
4 northwest of the country to the region of Central Greece (Figure 1B). The study
5 area forms a mosaic of elevations and habitats, from deep canyons and low
6 altitudes to steep and high mountains. Elevations range from a couple of hundred
7 meters above sea up to the peak of Mount Smolikas at 2,637 m above sea level.
8 This wide range of elevations results in two major forest zones: A higher
9 elevation conifer zone, with tree species such as the endemic Greek fir (*Abies*
10 *cephalonica*), the European black pine (*Pinus nigra*) and the Bosnian pine (*Pinus*
11 *heldreichii*) and a lower elevation mixed broadleaf forest, which is dominated by
12 oak (*Quercus* sp.), beech (*Fagus* sp.) and fir (*Abies* sp.). The local fauna
13 includes other large and medium-sized carnivores, such as the grey wolf (*Canis*
14 *lupus*) and the golden jackal (*Canis aureus*), and intensity of human activity
15 ranges from areas with low human presence to major cities with populations >
16 50,000.

17 **Sampling methods**

18 Genetic sampling was based on the natural marking and rubbing behavior of
19 bears (Green & Mattson, 2003) on wooden poles of the telephone and electricity
20 network (hereafter power poles) in Greece (Karamanlidis *et al.*, 2007) and
21 therefore we did not use any attractant to lure bears to the sampling stations. In
22 the preparatory phase of the study (2002-2006) we inspected and evaluated the
23 suitability of power poles as non-invasive genetic sampling stations according to
24 predefined criteria that considered among others marking and rubbing frequency

1 and intensity, type of habitat, vegetation density and intensity of human activity
2 (Karamanlidis, 2008); then we conducted a pilot study that confirmed the
3 suitability of power poles for the non-invasive genetic monitoring of brown bears
4 (Karamanlidis *et al.*, 2010).

5 For the purposes of this study and following the inspection and evaluation of
6 4147 power poles in the study area we selected 171 and placed barbed-wire hair
7 traps (Kendall & McKelvey, 2008) on them, thus creating four different sampling
8 areas (i.e., Vitsi - Varnoundas, and Northern, Central and Southern Pindos)
9 (Figure 1B, Table 1). We selected location and size of the sampling areas so as
10 to cover the maximum of the core range of the species in the Pindos mountain
11 range; sampling areas were separated either by distance or/and geo-
12 morphological features (e.g. extensive plains and valleys) and human
13 infrastructure (e.g. major highways). We calculated the size of the sampling
14 areas by drawing a buffer zone of 5.86 km around the Minimum Convex Polygon
15 (MCP) defined by the outermost sampling stations in each study area; 5.86 km
16 was the mean distance between all the individual bear recaptures recorded
17 during the study. We carried out systematic sampling efforts in all four sampling
18 areas from October 2007 to October 2010, when poles were inspected and hair
19 samples were collected monthly.

20 **Figure 1**

21 **Figure 1** A) Map of southeastern Europe. The shaded areas indicate the
22 approximate geographic range of brown bears in the region. B) Geophysical map
23 of Greece indicating the location of the study area. C) Map of Greece indicating
24 the location of four sampling areas for the non-invasive collection of brown bear

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

3 **Table 1** Descriptive statistics of four sampling areas for the non-invasive
4 collection of genetic samples from brown bears in Greece (2007–2010).

Sampling area	Location	Sampling area size (km²)	Sampling stations (N)
Vitsi – Varnoundas	N: 40.6935, E: 21.3192	1270.39	40
Northern Pindos	N: 40.3707, E: 21.0907	1066.22	45
Central Pindos	N: 39.9197, E: 21.4054	999.72	47
Southern Pindos	N: 39.6305, E: 21.3711	954.33	39
Total		4290.66	171

5 **Genetic Methods**

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

15 To determine individual identity each sample was genotyped at the
16 microsatellite loci G1D, G10J, G10L (Paetkau & Strobeck, 1994, Paetkau,
17 Shields & Strobeck, 1998), G10C, G10P (Paetkau *et al.*, 1995), MU51 and MU59

1 (Taberlet *et al.*, 1997). Gender identification was established through the analysis
2 of the amelogenin gene (Ennis & Gallagher, 1994). Up to 10 additional loci were
3 analyzed for ≥ 1 sample from each individual to enable more detailed population
4 genetic analyses. These extended genotypes were used to confirm differences
5 between individuals with similar 7-locus genotypes.

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

16 Mixed samples (samples with hair from >1 bear) were reliably identified by
17 evidence of ≥ 3 alleles at ≥ 1 locus (Roon *et al.*, 2005). To minimize genotyping
18 errors in the final data set, low-quality and putatively mixed samples were
19 excluded from further analyses (Paetkau 2003). Genotypes were replicated for all
20 1) individuals identified in one sample, 2) pairs of individuals that differed at only
21 1 or 2 loci (1- and 2-mismatch pairs) and 3) pairs of individuals that differed at 3
22 loci when ≥ 1 locus was consistent with allelic dropout. Test for allelic dropout,
23 presence of null alleles, and scoring errors caused by stutter peaks were

1 performed with Micro-Checker version 2.2.3 (van Oosterhout, Hutchinson & Wills,
2 2004).

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

11 **Estimating Abundance and Density**

12 To estimate abundance, we used the Jolly-Seber (JS) CMR modeling framework
13 that allows accounting for detectability less than one and dealing with open
14 populations, i.e., systems in which births and deaths occur (Jolly, 1965, Seber,
15 1965). JS models provide estimates of abundance, probabilities of survival,
16 detection and recruitment [*sensu* (Schwarz & Arnason, 1996) i.e., the probability
17 that an animal from the hypothetical super-population would enter the population
18 on a particular time interval]. We analyzed each of the four regions separately.
19 We considered time and sex effects as possibly explaining environmental and
20 individual variation in these demographic rates, entering in an additive or
21 interactive way in the model, which led to a total of 125 candidate models. To
22 select among these models, we used the Akaike's Information Criterion corrected
23 for small sample size (AICc) and considered the model with the lowest AICc
24 value as being the model that best balanced bias and precision (Burnham &

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

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

13 Although we considered a potential effect of sex on the detection probability,
14 other sources of individual heterogeneity might go undetected in the present
15 analysis, which could lead to severe bias in abundance estimates (e.g.,
16 (Cubaynes *et al.*, 2010b). This might be for example due to individuals being
17 more active than others at the proximity of power poles, hence making them
18 more capturable. To assess whether some individual heterogeneity was present
19 besides gender effects, we compared for each of the four study areas the model
20 best supported by the data to its counterpart, incorporating heterogeneity in the
21 detection probability. To do so, we considered finite-mixture JS models (Pledger,
22 Pollock & Norris, 2010) that extend standard JS models by assuming that the
23 animals come from different classes of detection, although we do not know which
24 class each individual is from. Heterogeneous JS models were fitted using the R

1 package 'hetage' that is available from <http://homepages.ecs.vuw.ac.nz/~shirley/>.
2 To compare homogeneous and heterogeneous models, AIC could not be used
3 because of the violation of regularity conditions (e.g. (Gimenez & Choquet, 2010)
4 in a CMR context), and we resorted to a likelihood ratio test that was distributed
5 under the null hypothesis of homogeneous detection probabilities as a 50:50
6 mixture of chi-square distributions with 0 and 1 degrees of freedom (Self & Liang,
7 1987). Note that, to get the P-value of this test, using the mixture corresponds to
8 halving the P-value from using the standard chi-square distribution with 1 degree
9 of freedom.

10 We calculated bear density using the sampled area size of each sampling
11 network respectively (i.e., Vitsi – Varnounds: 1270.39 km²; Northern Pindos:
12 1066.22 km²; Central Pindos: 999.72 km²; Southern Pindos: 954.33 km², Table
13 1). Less than 3% of the study areas were not suitable habitat for bears (e.g.,
14 lakes, rivers and human settlements); therefore we retained these areas in the
15 sampled area and density calculations.

16 **Results**

17 **Sampling Effort**

18 From 2007-2010, we conducted 4422 inspections to the 171 sampling sites and
19 collected 860 hair samples (Table 2). We collected bear hair at 92.25% of all hair
20 traps (i.e., Vitsi – Varnoundas: 95%; Northern Pindos: 100%; Central Pindos:
21 95%; Southern Pindos: 79%) during 25.75% of all power pole visits (i.e., Vitsi –
22 Varnoundas: 30%; Northern Pindos: 34%; Central Pindos: 26%; Southern
23 Pindos: 13%).

24 **Genotypic Success, Marker Power, and Quality Control**

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
 8 genotypes plus gender assignment.

9 **Table 2.** Descriptive statistics of efforts at four sampling areas in Greece (2007 -
 10 2010) for the non-invasive collection of brown bear hair samples

Sampling area	Visits	Bear presence	Hair samples collected	Females identified	Males identified	Total N identified	N of animals recaptured
Vitsi Varnoundas	– 1115	358	215	13	39	52	28
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	4422	1262	860	61	150	211	102

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

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

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

Marker	H_E^a	H_O^a	A^a	P_{ID}^a	P_{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				5.299e-07	0.002

^a H_E = expected heterozygosity; H_O = observed heterozygosity; A = number of alleles; P_{ID} = probability of identity; P_{SIB} = probability of identity among siblings

5 **Abundance and density estimation**

6 The standard assumptions of the JS model were valid as showed by the
 7 goodness-of-fit tests that were non-significant for all regions ($\chi^2_3 = 2.45$, p-value =
 8 0.49 for Vitsi – Varnoundas; $\chi^2_4 = 3.58$, p-value = 0.47 for Northern Pindos; $\chi^2_7 =$
 9 0.47, p-value = 0.99 for Central Pindos; $\chi^2_2 = 0.71$, p-value = 0.70 for Southern
 10 Pindos). Model selection showed contrasted effects between regions (Table 4).
 11 Survival was constant in Vitsi - Varnoundas and Southern Pindos and sex-
 12 specific in Central and Northern Pindos with the addition of time in the latter.
 13 Recruitment was constant in all regions but Northern Pindos in which it was sex

1 and time dependent on an additive scale. Detection was constant in all regions
 2 but Vitsi – Varnoundas in which it was sex-specific. There was substantial
 3 uncertainty regarding the best model to select for all regions, with several models
 4 within the range $\Delta AICc < 2$, which motivated the use of model-averaged
 5 abundance estimates (see below). We did not detect extra heterogeneity in the
 6 detection probability ($\chi^2_1 = 2.5$, p-value = 0.06 for Vitsi – Varnoundas; $\chi^2_4 = 2.6$,
 7 p-value = 0.31 for Northern Pindos; $\chi^2_7 = 8.5$, p-value = 0.15 for Central Pindos;
 8 $\chi^2_1 = 0.6$, p-value = 0.22 for Southern Pindos).

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

Region	Model	k	AICc	$\Delta AICc$
Vitsi- Varnoundas	$\phi(.) p(s) b(.)$	5	95.17	0.00
	$\phi(s + t) p(.) b(s)$	7	96.49	1.34
	$\phi(s) p(.) b(s)$	6	97.51	2.36
	$\phi(t) p(s) b(.)$	6	97.51	2.37
	$\phi(.) p(s) b(s)$	6	97.54	2.39
	$\phi(.) p(s) b(t)$	6	97.57	2.42
	$\phi(s) p(s) b(.)$	6	97.58	2.43

	$\varphi(s) p(.) b(.)$	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
	$\varphi(s) p(t) b(s + t)$	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
	$\varphi(s) p(.) b(t)$	8	128.82	2.79
	$\varphi(s) p(t) b(t)$	9	129.09	3.06
	$\varphi(s) p(t) b(s)$	9	129.41	3.38
	$\varphi(s + t) p(t) b(s + t)$	9	129.95	3.92
Central Pindos	$\varphi(s) p(.) b(.)$	8	143.03	0.00
	$\varphi(s) p(.) b(t)$	8	143.64	0.61
	$\varphi(.) p(s) b(.)$	9	143.77	0.74
	$\varphi(s + t) p(.) b(.)$	7	144.56	1.54
	$\varphi(.) p(.) b(.)$	10	144.73	1.71
	$\varphi(s) p(.) b(s)$	9	144.75	1.72
	$\varphi(s + t) p(.) b(t)$	7	144.76	1.73
	$\varphi(s) p(.) b(s + t)$	10	144.82	1.79
	$\varphi(.) p(s) b(t)$	8	144.82	1.79
	$\varphi(s) p(s) b(.)$	11	144.83	1.80
Southern Pindos	$\varphi(.) p(.) b(.)$	4	55.05	0.00
	$\varphi(s) p(.) b(.)$	5	55.14	0.09
	$\varphi(.) p(s) b(.)$	6	57.84	2.78
	$\varphi(t) p(.) b(.)$	7	57.96	2.91
	$\varphi(.) p(.) b(s)$	5	57.98	2.93
	$\varphi(s) p(s) b(.)$	6	58.00	2.95
	$\varphi(.) p(.) b(t)$	6	58.04	2.99
	$\varphi(s + t) p(.) b(.)$	5	58.28	3.23
	$\varphi(s) p(.) b(s)$	8	58.39	3.34
	$\varphi(s) p(.) b(t)$	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).

5 **Table 5.** Abundance estimates from CMR analysis of the brown bear populations
 6 in Greece using the Jolly-Seber modeling framework. For each of the four
 7 regions (i.e., Vitsi - Varnoundas and Northern, Central and Southern Pindos), the
 8 time and sex-specific population size estimate, as well as the lower (LCL) and
 9 upper (UCL) confidence limits of the 95% confidence interval obtained from
 10 model averaging are given.

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

		UCL	122.8	113.6	102.1
		value	22.2	14.5	11.4
	female	LCL	12.3	6.5	3.5
		UCL	40.1	32.4	37.2
		value	41.9	34.2	28.7
Central Pindos	male	LCL	26.4	21.2	13.9
		UCL	66.6	55.0	58.9
		value	64.1	48.6	40.1
	pooled	LCL	39.1	28.9	18.1
		UCL	105.0	81.9	88.6
		value	2.1	1.0	0.9
	female	LCL	0.7	0.2	0.2
		UCL	6.0	4.8	3.8
		value	11.6	8.2	7.0
Southern Pindos	male	LCL	7.6	5.4	3.8
		UCL	17.5	12.7	12.1
		value	13.6	9.3	7.7
	pooled	LCL	8.3	5.5	4.1
		UCL	22.3	15.6	14.4

1 Discussion

2 We present the first rigorous estimate of brown bear abundance and density in
3 the western range of brown bears in Greece. By using genetic data from hair
4 samples collected from poles of the electricity and telephone network, an
5 approach that has not been implemented in this scale before, we obtained
6 precise population estimates despite fairly low capture and recapture rates. The
7 remarkable consistency of our estimates between years (especially the years
8 2008 and 2009) lends credibility to our results.

9 There are several assumptions that need to be checked to use the Jolly-Seber
10 model and obtain reliable abundance estimates. First, we assumed that there

1 was no immigration or emigration (i.e., closure assumption) in the four regions
2 we studied. This was not entirely true as several individuals moved from region
3 XX to region XX during the study period. A modelling option would have been to
4 use multisite JS models (e.g., (Lebreton *et al.*, 2009) instead of considering the
5 region as a factor, hence allowing the estimation of movement between regions.
6 However, with so few individuals actually making the transitions between regions,
7 fitting multisite JS models would be numerically unstable with movement
8 parameters estimated on the boundary (Gimenez *et al.*, 2005) and identifiability
9 issues (Gimenez, Choquet & Lebreton, 2003). Add here a sentence on how we
10 should interpret our abundance estimates consequently. A second assumption
11 underlying the application of JS models is that all animals, marked or unmarked,
12 should have the same capturability (i.e., homogeneous capturability assumption).
13 Here, there was no reason to believe that the use of poles to “capture” individuals
14 would distinguish between marked and unmarked individuals. Third, the duration
15 of the sampling period should be short in comparison to the time between
16 sampling occasions. In our study, most of the observations were made over eight
17 months, which was considered a short enough with regard to the yearly time
18 interval we used between sampling periods as bear survival was high (Hargrove
19 & Borland, 1994). Fourth, and probably the most crucial assumption, detection
20 probabilities were assumed homogeneous between individuals, heterogeneity if
21 present and ignored being source of strong bias in the abundance estimates
22 (Cubaynes *et al.*, 2010a). We found a sex effect for the Vitsi – Varnoundas
23 population, males being more detectable than females (0.18 [SE=0.12] for
24 females and 0.38 [SE=0.08] for males), and this difference was accounted for
25 when calculating abundance estimates. We also tested for extra individual

1 heterogeneity not explained by differences between males and females, but did
2 not find any, which suggest that the risk of bias in abundance estimates was
3 avoided. Last but not least, it is usually assumed that individuals retain their mark
4 throughout the study and that these marks are read correctly. When interpreted
5 in the context of DNA marking, these two assumptions refer to data quality and
6 the possibility of genotyping error. Last but not least, issue of the coverage of the
7 poles.

8 **Management implications**

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

13 **Conclusions**

14 **Acknowledgements**

15

16 **References**

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