available at www.sciencedirect.com

SCIENCE ⓓ DIRECT®

journal homepage: www.elsevier.com/locate/biocon

ELSEVIER

BIOLOGICAL CONSERVATION

An evaluation of field and non-invasive genetic methods to estimate brown bear (*Ursus arctos*) population size

Knut Håkon Solberg[a], Eva Bellemain[a,b], Ola-Mattis Drageset[a], Pierre Taberlet[b], Jon E. Swenson[a,c,*]

[a]Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Box 5003, NO-1432 Ås, Norway
[b]Laboratoire d'Ecologie Alpine (LECA), Génomique des Populations et Biodiversité, CNRS UMR 5553, Université Joseph Fourier, BP 53, F-38041 Grenoble Cedex 9, France
[c]Norwegian Institute for Nature Research, Tungasletta 2, NO-7485 Trondheim, Norway

ARTICLE INFO

ABSTRACT

Estimates of population size and density are essential for successful management and conservation of any species. Although there are a variety of methods available for estimating abundance and density of populations, most studies rely on only one estimator and very few studies have compared and critically evaluated the adequacy and the cost of these methods. We used the brown bear (*Ursus arctos*) in south-central Sweden to compare the performance of three different methods of estimating population size, including methods based on conventional field data as well as on non-invasive genetic data. The method based on observations of females with cubs underestimated the true population size, as the estimates were below the number of unique genotypes determined from faecal data inside the study area. The best traditional method was based on observations of bears from a helicopter. The genetic method using the closed population MARK estimator, as recommended in a previous study, seemed to perform the best. We conclude that approximately 223 (188–282) bears were present in our 7328 km² study area during 2001 and 2002 and suggest that this hunted brown bear population has been relatively stable for about ten years. The non-invasive genetic method was less expensive than the most reliable traditional field method (a CMR method based on observations of bears from a helicopter), and preferable from an ethical point of view. We recommend that future studies using non-invasive genetic methods based on collected faecal samples should aim at collecting 2.5–3 times the number of faecal samples as the "assumed" number of animals.

1. Introduction

Ecological theory and wildlife management often depend on reliable estimates of population size and density (Smallwood and Schonewald, 1998). Estimating population size is not an easy task, especially for elusive and endangered species (Wesley et al., 2000). Traditional field methods of population size estimates are generally based on distance sampling, including direct counts or transect sampling (Buckland et al., 1993) or on capture-mark-resight/recapture (CMR) methods, including aerial surveys, hunter CMR or camera CMR (Seber, 1982). However, capture methods involve dangers of injury or death to the animal (Arnemo et al., in press) and this ethical aspect deserves consideration, especially for endangered populations

(Bekoff and Jamieson, 1996). Non-invasive genetic methods have recently been used to estimate population sizes (Kohn et al., 1999; Banks et al., 2002, 2003; Eggert et al., 2003; Wilson et al., 2003; Flagstad et al., 2004; Bellemain et al., 2005). The use of genetic samples, such as hairs or faeces, allows individual identification, without the need to see or disturb the animal (Taberlet et al., 1999). Technical problems associated with this method (due to low quality and quantity DNA samples) are now well understood (Taberlet et al., 1999; Smith et al., 2000), and methods have been developed to overcome those limitations (reviewed in Paetkau, 2003; Piggott et al., 2004).

However, to our knowledge, very few studies have compared several methods of population size estimation, including field and genetic methods in order to critically evaluate the reliability and adequacy of methods used. Moreover, the costs of each method, in terms of time, money and ethics, are not generally reported.

The brown bear (*Ursus arctos*) is a typical elusive species for which population sizes are difficult to estimate (Kolstad et al., 1986; Kendall et al., 1992; Eberhardt and Knight, 1996). Both male and female brown bears are secretive, have large home ranges (Dahle and Swenson, 2003a), and maximum movements of up to 42 km/24 h have been recorded in Scandinavia (Wabakken and Maartman, 1994). Thus, it is difficult to develop standard methods of population size estimation with acceptable levels of precision and accuracy.

We studied the Scandinavian brown bear population, which represents a characteristic example of population bottleneck and subsequent population expansion (Swenson et al., 1995, 1998). Using traditional field methods, the Swedish brown bear population size was estimated to be about 300 individuals in 1942 (Selander and Fries, 1943) and about 1000 bears in 1996 (Sandegren and Swenson, 1997). However, there is a great need to determine the size of the present population (Naturvårdsverket, 2003).

Bellemain et al. (2005) compared population estimates of Scandinavian brown bears from four methods using non-invasive genetic data from faecal sampling conducted by volunteer hunters and others during two consecutive years in a 49,000 km² area in southern Sweden, as well as the same study area as described in this paper. They used two different equations for rarefaction indices (Kohn et al., 1999; Eggert et al., 2003), one Lincoln Peterson (LP) estimator (Seber, 1982) considering radio-marked bears as the capture group and the genetic faecal samples as the recapture group, and closed population models in MARK (White and Burnham, 1999), also based on the CMR principle. The most reliable estimates were considered to be the estimates from the LP estimator, but the MARK method also performed well. Those authors did not evaluate field methods.

The goal of the present study was to compare the performance of different population size estimators derived from both conventional field data and non-invasive genetic data, in order to recommend a method for future management of elusive animal populations, specifically for the current management and conservation of the bear population in Sweden. The non-invasive genetic method was based on the MARK closed population estimator, as recommended by Bellemain et al. (2005). Field methods were based on (1) observations of females with cubs and (2) a CMR study based on observations from a helicopter of females in oestrus in company with radio-marked adult males. Each estimator was assessed for its performance by comparing it to the minimum population size, represented by the number of unique genotypes identified among the fecal samples inside the study area, as well as to the LP estimates from Bellemain et al. (2005), regarded as the most precise and accurate estimate they obtained. In addition, we evaluated the cost of the best performing field method (the helicopter method) and the non-invasive genetic method, in terms of time and money, in order to give recommendations for management.

2. Methods

2.1. Study area

In order to meet the assumption of closure for the helicopter capture–recapture method, we defined the study area as the composite 100% minimum convex polygon (MCP) of all the positions of marked females in oestrus during the mating season, in both 2001 and 2002 (7328 km² ; Fig. 1). The landscape in the study area is mainly forested with Scots pine (*Pinus sylvestris*) (66%) and Norway spruce (*Picea abies*) (32%). Various deciduous trees, such as common birch (*Betula pubescens*), silver birch (*Betula pendula*), aspen (*Populus tremula*) and grey alder (*Alnus incana*), are common in early successional stages of the forest. The forest in the study area is intensively managed by clear-cut harvesting, and clear-cuts are a relatively important component of the forest landscape. The terrain is dominated by gently rolling hills, with elevations around 200 m.a.s.l.

2.2. Population size estimates using field data

2.2.1. Observations of females with cubs from the public
We used observations of females with cubs reported by the public during the 2001 and 2002 mating seasons (1 May–30 June; Dahle and Swenson, 2003a) to estimate population size. These observations were verified by volunteers responsible for investigating sightings of large carnivores for the Swedish Association for Hunting and Wildlife Management. However, volunteers could not identify individual family groups and thus not document the number of times a known family group was reported.

The model behind this method is based on a study of daily movements of radio-marked females with cubs inside the study area during the mating seasons in 1998 and 1999 (Kristoffersen, 2002). Observations from the mating season were used because most females with cubs restrict their movements during this period, whereas there are longer movements and more variation in daily movements among females with cubs in late summer (Kristoffersen, 2002). Radio-telemetry was used to determine the location of a female with cubs every 24 (±6) hours. Then, the shortest linear distance between observations was calculated to estimate rates of daily movement. Kristoffersen (2002) plotted days between observations against distance between observations and drew the nonlinear regression line that gave the minimum sum of squares. This resulted in an asymptotic curve.
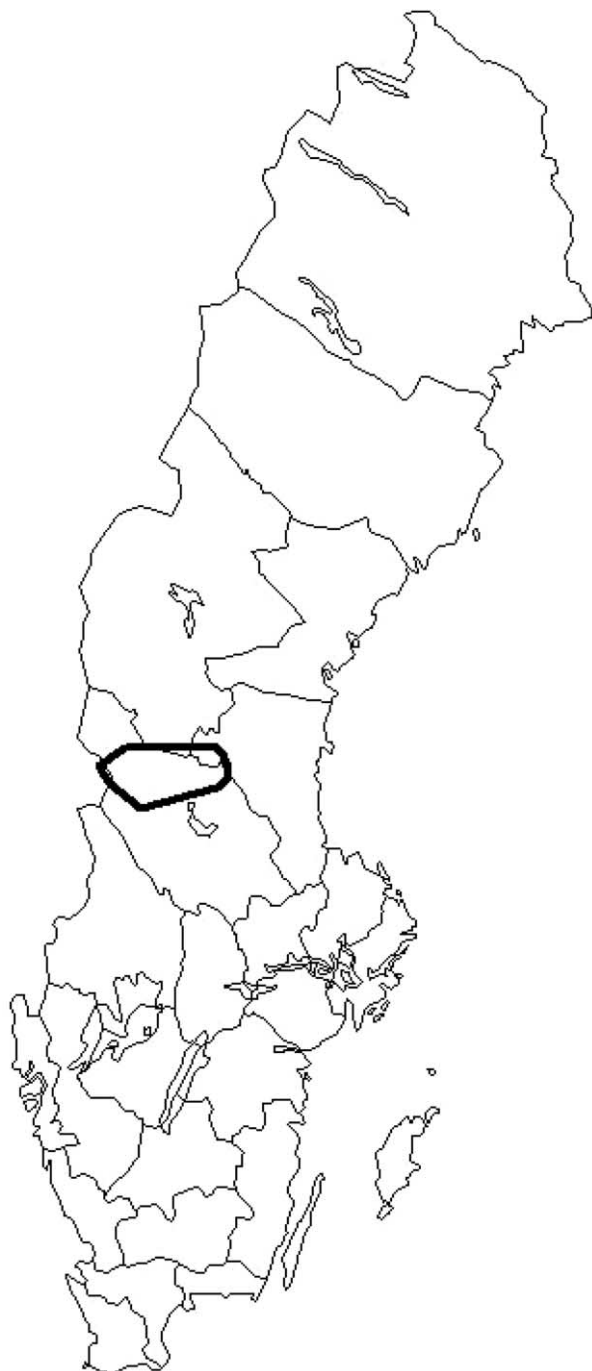
Fig. 1 – The study area boundaries in Sweden. The study area is 7328 km².

We used the upper 95% confidence interval of the regression equation in our calculations, which gave the maximum distance (D_{max}) a family group would have been expected to move in X days, with 95% certainty:

$$D_{max} = (9.77 \times X)/(5.06 + X),$$

where:
D_{max} is the maximum distance moved in km,
X = time between observations in days,

9.77 and 5.06 are constants from the regression equation representing the upper 95% confidence interval.

Two observations of females with cubs separated by X days had to be further apart in kilometers than D_{max} to be regarded as different family groups. Pairs of observations had to be less than 20 days apart in time to use this equation; after 20 days the variance in traveling distances becomes too high to use it. However, we extended the model up to 60 days to include observations from the entire mating season. The D_{max} between 20 and 60 days was defined as the longest distance between two radio-telemetry fixes of each marked female with cubs during the mating season, averaged over all marked females inside the study area (9.25 ± 1.06 km). We estimated the mean home range size (100% MCP) of females with cubs during the mating season in this area to be 82 km², which corresponds to a radius of 5.1 km. This gives us further confidence that the defined D_{max} between 20 and 60 days was a conservative approach. Observations of family groups that were less than D_{max} away from another family group were subtracted from the total number of observations to obtain a minimum estimate of females with cubs inside the study area. Although this model avoided duplicate counts of the same family group, it did not allow for identification of different family groups seen near each other and therefore gave an underestimate of the true number of females with cubs. We calculated the total population size and density inside the study area based on survival rates and reproduction rates of radio-marked bears (Appendix 1), using the equation described in Appendix 2a. The confidence intervals associated with the demographic parameters were used to calculate the confidence intervals for total population size, which allowed us to consider parameter uncertainty in the estimates.

2.2.2. CMR from a helicopter

We used the method of Swenson et al. (1994) to estimate the number of females in oestrus during the mating season, but we made all observations from a helicopter instead of from small fixed-wing planes and the ground. Eleven radio-marked adult male bears ($\geqslant 5$ years) with radio-transmitters were monitored in both 2001 and 2002 to locate females in oestrus. Aerial surveys were carried out three times a week from mid May until 1 July, resulting in 15 surveys in 2001 and 14 in 2002. Three persons, including the pilot, conducted each aerial survey. Two teams with different responsibilities were formed, each equipped with a radio-receiver (Telonics TR-4, Telonics). Team 1 (the pilot and one field technician) surveyed the area for adult males, whereas team 2 (one field technician) scanned for other radio-marked bears in the area. There was no communication between the teams regarding the presence or absence of additional radio-marked bears in order to avoid bias in search time when a marked female was present. The pilot carefully circled the male bears with the helicopter until the male was observed. This careful approach was important to avoid splitting of the male and a potential female in oestrus. If an additional bear was sighted, team 1 used telemetry equipment to determine whether the bear was radio-marked or not. Marked females that were known to be with a male, based on radio-telemetry data, but not visually observed, were not included in the survey sample.

We used the program NOREMARK (White, 1996a) and the Minta–Mangel estimator (Minta and Mangel, 1989; White, 1996b) to estimate the number of oestrus females on the study area in 2001 and 2002. This bootstrap estimator is based on the frequency of resightings of marked individuals, and the total sightings of unmarked individuals. The model assumes a sample drawn with replacement, so that marked animals might be seen more than once on a survey (Schwartz and Seber, 1999). We calculated 95% confidence intervals of oestrus females, based on the variance of the resighting frequencies. The Minta–Mangel model requires three model parameters: (1) number of available marked females in oestrus, (2) number of sightings of each marked female in oestrus, and (3) total number of unmarked females seen with males. The model assumptions were: (1) population closure, (2) no animals lost their radio-transmitters during the survey, and (3) correct mark identification. The closure assumption was met (see Section 2). No females lost their radio-transmitters during the study, and all the marked females had radio-transmitters with individual frequencies, thus assumptions (2) and (3) were met. Observations of females in the company of males outside this area were excluded from the sample. We used the equation described in Appendix 2b, based on long-term data on marked bears survival rates and reproduction (Appendix 1), to calculate the total population size in the study area. As 3-year-old females come into oestrus in this population (Swenson et al., 1994; Dahle and Swenson, 2003b), we considered all 3-year-old females to be adults in these calculations. The confidence intervals associated with the demographic parameters, as well as the confidence intervals from the Minta–Mangel estimator, were used to calculate the confidence intervals for total population size. This allowed us to consider parameter uncertainty in the estimates.

2.3. Population size estimates using non-invasive genetic data

A more detailed description of this method is given in Bellemain et al. (2005).

2.3.1. Faecal sampling
The faecal sampling used in this study (7328 km^2 area) was part of the larger faecal sampling conducted in autumn 2001 and 2002 in Dalarna and Gävleborg counties (Bellemain et al., 2005). All faecal samples (independently of their age) were collected opportunistically when found by cooperating hunters hunting moose (*Alces alces*), volunteers and personnel from the Scandinavian Brown Bear Research Project, and preserved in 95% alcohol until DNA extraction. For each faecal sample, the sampling date, the location and geographical coordinates (Swedish grid) were recorded.

2.3.2. DNA extractions and typing
DNA extractions were performed using the Qiamp DNA Stool kit involving overnight digestion with proteinase K. Six microsatellite loci (Mu10, Mu23, Mu50, Mu51, Mu59, G10L) and one specific sex primer (SRY; Bellemain and Taberlet, 2004) were amplified using the multiplex preamplification method (Piggott et al., 2004) and following the protocol described in Bellemain and Taberlet, 2004. This method allows one to maximise the number of samples that contain the critical threshold amount of DNA for accurate genotyping. Each amplification was repeated four times, as a preliminary study determined that reliable genotypes were obtained after this number of replicates (multi-tubes approach, Taberlet et al., 1996). A high genetic diversity was previously revealed in the Scandinavian brown bear population (Waits et al., 2000). In this study, the number of alleles per locus ranged from six to nine, with a mean observed heterozygosity of 0.70. The gels were analysed using GENEMAPPER version 3.0 software package. We computed the probability of identity, i.e. the overall probability that two individuals drawn at random from a given population share identical genotypes at all typed loci, between unrelated individuals (PI; Paetkau and Strobeck, 1994) and between siblings (PIsibs; Waits et al., 2000). We calculated the genotyping error rate (due to allelic dropout, false alleles or contaminations; Taberlet et al., 1996) by randomly repeating, another four times, 5% of the amplifications, and comparing the first and second typings. Genotypes from different samples were considered to represent an identical individual when all the alleles at all loci were identical. However, when there was only one mismatch for one allele at one locus, we considered the two samples as belonging to the same individual.

2.3.3. Population size estimates from the MARK closed population estimator
The MARK closed population estimator was chosen to estimate population size from non-invasive genetic data, as recommended by Bellemain et al. (2005). This estimator is also based on the principle of capture-mark-recapture where individual bears are "captured" and "recaptured" through their genotyping identification in the faecal sampling. It consisted of grouping identical multilocus genotypes and compiling a capture and recapture history for each individual by dividing the data set into weekly capture periods (11 weekly periods for 2001 and 13 for 2002). If an individual had been captured more than once within the same capture period, only one capture was considered. Data were analysed as conventional CMR data using the closed-capture models of MARK (White and Burnham, 1999). The estimate from the best approximating model of the candidate set, based on AICc (Akaike's Information Criterion corrected for small sample size), was considered.

3. Results

3.1. Population size estimates using conventional field data

3.1.1. Observations of females with cubs
The total number of reported and verified observations of females with cubs inside the study area during the mating season (Fig. 3) was three in 2001 and six in 2002, which resulted in estimates of two and five females with cubs in 2001 and 2002, respectively. The total population size estimates were 27 (21–35) and 66 (52–88) bears, respectively (Table 1, Fig. 2). The number of marked females with cubs inside the study area, which represented the known minimum number at the end of the mating season, was seven in 2001 and 10 in 2002. In other words, this method underestimated the brown

Table 1 – Estimates of the total number and density (bears/1000 km²) of brown bears inside the study area in south-central Sweden in 2001 and 2002 from the different methods

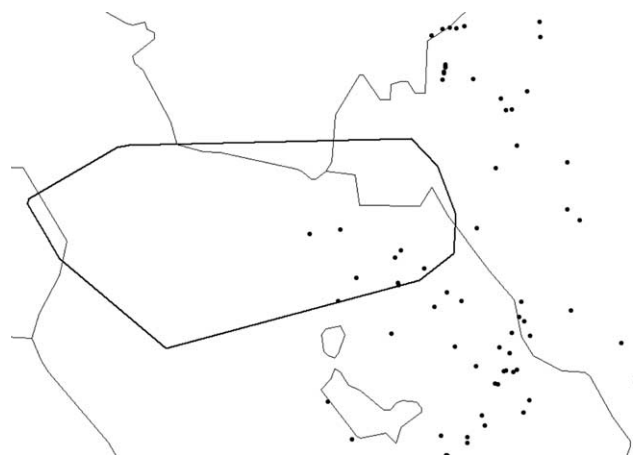| Method	2001 Size	2001 Density	2002 Size	2002 Density
Observation of females with cubs	27 (21–35)	3.6 (2.9–4.8)	66 (52–88)	9.1 (7.2–12.1)
CMR from helicopter	179 (96–389)	24.5 (13.1–5.1)	149 (128–256)	20.4 (17.5–35.0)
MARK program	223 (188–282)	30.4 (25.7–38.5)	157 (119–227)	21.4 (16.2–31.0)
Lincoln–Petersen	219 (157–314)	29.9 (21.4–42.8)	204 (136–272)	27.8 (18.6–37.1)
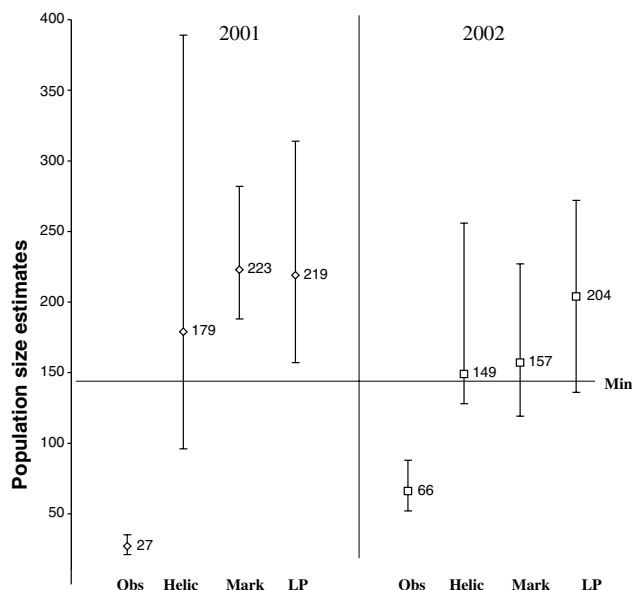
Fig. 2 – Population size estimates of brown bears inside the study area in south-central Sweden in 2001 and 2002 from the different methods. Error bars show 95% confidence intervals. Obs – estimates based observation of females with cubs; helic – CMR helicopter estimates (based on observation of oestrus females); mark – MARK program estimates (non-invasive genetic method); LP – Lincoln-Petersen estimates (genetic method); min – minimum population size represented by the number of unique genotypes identified in the fecal sampling in 2001.

Fig. 3 – Verified observations of females with cubs reported by the public in 2001 and 2002 in Dalarna and Gävleborg counties in south-central Sweden, within and outside the limits of our study area.

bear population considerably both years. Outside the study area, there were many more verified observations, especially to the east (Fig. 3).

3.1.2. CMR helicopter survey
We made a total of 89 and 105 observations of males in 2001 and 2002, respectively. Male–female pairs were observed 19 times in 2001 (nine observations of marked females and 10 of unmarked females) and 23 times in 2002 (20 observations of marked females and three of unmarked females). The increase in the proportion of marked females was due to a concentrated effort to capture adult females in the area. Females were seen with an adult male during 21% and 22% of the male observations in 2001 and 2002, respectively. We failed to observe nine marked females that were known to be present with marked males, based on telemetry data, in 2001, and seven marked females in 2002. This was due to dense cover or splitting of the male and the female before the male was

observed. Our total population size estimates were 179 (96–389) bears in 2001 and 149 (128–256) bears in 2002 (Table 1, Fig. 2).

3.2. Population size estimates from non-invasive genetic data

In 2001, 353 faeces (70% of the collected faeces) were successfully amplified for 6–7 loci (including the sex locus) and 146 unique genotypes were identified. In 2002, 154 faeces (80% of the collected faeces) were successfully amplified for 6–7 loci and 81 unique genotypes were identified. We ensured a high quality and reliability of the genetic data, with a calculated genotyping error rate below 2%; in addition, the genetic results were geographically consistent (Bellemain et al., 2005). The PI among the seven amplified loci was low (PI = 1.38×10^{-6}; PIsibs = 4.52×10^{-3}), which allowed us to identify each individual reliably. For both years, the best approximating model of the MARK closed-capture candidate model set included heterogeneity and temporal variation in detection probabilities. The population size estimates, derived from this model, were 223 bears in 2001 and 157 in 2002, with relatively low confidence intervals (Table 1, Fig. 2).

4. Discussion

There are a variety of methods available for estimating abundance and density of wild animal populations. To our

knowledge, our study is one of the first to simultaneously compare field and genetic methods.

In our study population of brown bears, we found that population size estimates varied greatly by method (Table 1, Fig. 2). It is difficult to make a direct comparison of estimates of population size derived from different methods. One major problem is the underlying assumptions of the estimation models. This is especially true for the assumption of geographic closure, which is rarely met in natural populations (Arnason et al., 1991). Due to the conservative approach in defining our study area, we have good reasons to believe that the assumption of population closure was met for the CMR field method. However, it is likely that this assumption was violated for the other methods considered. A violation of the closure assumption can result in biased population size estimates (White et al., 1982; McCullough and Hirth, 1988; Hallet et al., 1991; Castley et al., 2002).

Hereafter we discuss the performance of the field and genetic methods and compare the estimates obtained with the number of unique genotypes found in 2001, 146 bears, which can be considered as the minimum population size. We consider this number rather than the 2002 number because the 2002 sampling was much lower and not randomly distributed. This minimum population size estimate is low compared to the LP estimates from the same area (Bellemain et al., 2005). The LP estimates are based on relatively large sample sizes (146 unique feces genotypes in 2001 and 81 in 2002), and consistent among the two years with estimates of 219 (157–314) bears in 2001 and 204 (136–272) bears in 2002. In addition, a large proportion of the marked bears were identified in the faecal sampling in the study area (64% in 2001 and 49% in 2002), which is important to obtain unbiased population size estimates (Bartmann et al., 1987). Therefore, the LP estimates are probably the most precise and accurate estimates of the brown bears population size in the study area. However, the LP estimator is not generally applicable, because it requires that a relatively large proportion of the population is marked, which is rare in populations of elusive species like the brown bear.

4.1. Population size estimates using conventional field methods

4.1.1. Observations of females with cubs

Estimates based on verified observations of females with cubs reported by the public were lower than the minimum population size in the area (number of unique genotypes). Moreover, the number of observations verified was far less than the number of marked females with cubs on the study area in 2001 and 2002. Similar methods have been used to obtain minimum population size estimates of grizzly bears in Yellowstone National Park, USA, since 1976 (Knight et al., 1995; Eberhardt and Knight, 1996; Keating et al., 2002) and brown bears in the Cantrabian Cordilla, Spain since 1982 (Wiegand et al., 1998). Estimates based on observations are less expensive than methods involving capturing and handling of individuals and could therefore be applied more often, across larger areas, and for longer periods of time than, e.g. CMR techniques (Smallwood and Schonewald, 1998). If the studied species is relatively easy to detect, encounter rates may provide a reasonable index of variation in population size (Wesley et al., 2000). However, the low density and elusive behaviour of brown bears makes observations of females with cubs more suitable as an indicator of population trend, rather than a tool for estimating population size and density (Linnell et al., 1998). Others have indicated that further testing is necessary to determine the reliability of this method (Kendall et al., 1992; Kristoffersen, 2002). Mattson (1997) pointed out that all the parameters possibly influencing data collection (human effort, attitudes, awareness, distribution and abundance of bear food), and hence the estimate, could vary among years with little relationship to the number of females. Also, errors in identifying family groups, false observations (Elgmork et al., 1976) and changes in routines for validation of observations are all possible sources of bias in our data. Additionally, one could assume that reporting rates of observations are related to human population density. Our study area has an average of only about three people per km^2 and most of these people live around the edges and outside the study area (Fig. 4). Most
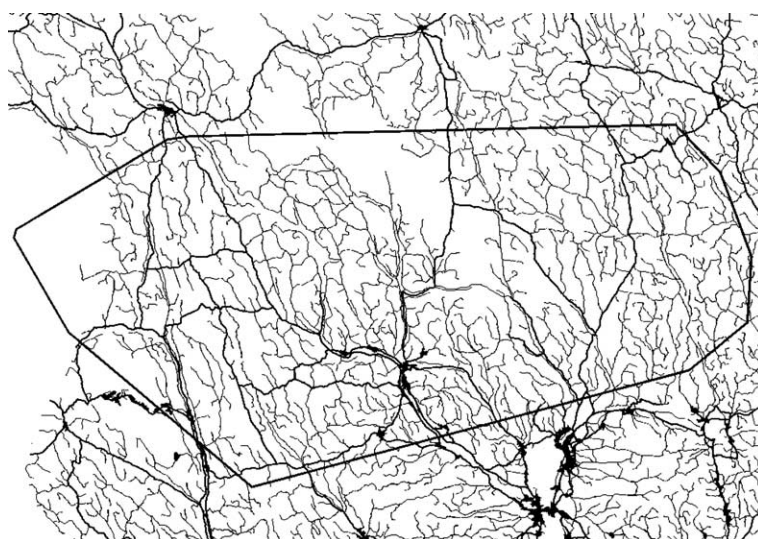
Fig. 4 – Human population inside and outside the study area. The lines represent roads, the black areas represent human villages and small towns.

observations were made outside the study area in areas with a lower density of bears and a higher human population than inside the study area (Fig. 4). More observations would be needed inside our study area to test the adequacy of the model. Thus better systems for collection and validation of observations of females with cubs could improve the utility of this method. Also, this method may be more suitable for the edges of expanding populations, where reported rates presumably are higher.

4.1.2. CMR helicopter survey

There was a high proportion (69%) of marked females among oestrus females observed with adult males from the helicopter in the study area. Bartmann et al. (1987) emphasized that it is important to have a high proportion (>45%) of marked animals in a population to obtain unbiased population size and density estimates. Thus, a CMR method should be appropriate to estimate the brown bear population in our study area. There was no variation in the proportion of times females were observed with marked males between 2001 and 2002 (0.21 and 0.22, respectively). However, we observed more unmarked oestrus females in 2001 than in 2002 (10 and 3, respectively). We underestimated the true number of females in oestrus inside the study area in 2002, as the population size estimate derived from this number was below the number of unique genotypes. The estimates of females in oestrus decreased from 2001 to 2002 and therefore the population size and density estimates decreased (Table 1, Fig. 2). However, we have no reason to believe that the population in our study area was decreasing and the difference between 2001 and 2002 was most likely due to small-sample bias, as our sample sizes both years were below 30 (White et al., 1982). This difference could also be the result of temporal variation in the true number of females in oestrus in our relatively small study area.

Estimates of population size based on various mark-recapture and mark-resight methods from small planes or helicopters have been carried out on many different bear populations (Miller et al., 1987; Swenson et al., 1994, 1995; Miller et al., 1997), as it is often the only method available for population size estimation of low-density carnivore populations (Greenwood et al., 1985). However, the results often have poor accuracy and low precision, due to small sample sizes (Neal et al., 1993; Krebs, 1999) and problems with meeting the underlying assumptions of the estimation models (White et al., 1982; McCullough and Hirth, 1988; Hallet et al., 1991; Castley et al., 2002).

4.2. Population size estimates using genetic methods

The genetic data proved to be reliable thanks to a low genotyping error rate, a high probability of distinguishing among individuals, and geographical consistency of the results (Bellemain et al., 2005). The MARK closed population estimator allows one to incorporate heterogeneity and temporal variation in detection probabilities and the confidence intervals were reasonably small. However, this estimator yielded a noticeably lower point estimate in 2002 than in 2001 (Fig. 2), although this was not statistically significant. There are two possible explanations for this. First, only 154 faecal samples

were genotyped in the study area in 2002, whereas 359 were genotyped in 2001. Secondly, individuals in 2002 had a significantly lower chance of being captured than in 2001 (each individual was captured 1.69 ± 1.13 (SE) times in 2001 against 1.40 ± 0.915 (SE) times in 2002; two-tailed t-test; $p = 0.04$). Consequently, many bears were missed in the 2002 genetic sampling, and we consider that the 2001 sampling gave more reliable estimates. Therefore, it seems reasonable to conclude that the number of bears in our study area was about 223 (188–282), or a density of 30 bears per $1000 \, km^2$, based on the 2001 MARK estimate. This was also very close to the LP estimates, 219 (157–314) in 2001 and 204 (136–272) in 2002 (Bellemain et al., 2005).

4.3. Analysis in terms of cost/benefit of each method

It is difficult to estimate size and density of brown bear populations. We have concluded that the two conventional field methods tended to underestimate the true population size. The most reliable field method was the helicopter CMR method, which is a dilemma, as it is very expensive and depends on the presence of radio-marked bears. However, the non-invasive genetic method also gave a reasonable estimate.

As a guide to managers who are considering estimating brown bear population sizes over large areas, we have calculated the actual costs of these two most reliable estimation methods, using 2004 prices. The most important point of these calculations is to compare the relative costs of the two methods. Of course, the actual costs in a given country will vary according to many factors, including cost levels for goods and wages, value-added taxes, and overhead costs. In our calculations, we have included the value-added taxes (25% in Sweden and 19.6% in France) on goods and services, but not on wages or mileage allowances. No overhead costs were included.

The costs for the non-invasive genetic estimate consisted of informing the hunters at both the central and local level and following this up, giving information to local media, sending out collection tubes, receiving them, placing ethanol in the tubes, checking the labeling, and entering the data into the data base. For the two years, costs were about EUR 3400 for travel costs (mostly to inform hunting groups prior to their participation), EUR 1850 for materials (freight, postage, ethanol, collection tubes), and EUR 7800 for 2.7 months wages for a person to carry out this work. The laboratory costs, including analysis and salaries, at a commercial laboratory would have been EUR 96 per sample, or about EUR 48,700 for 507 samples. Thus, for the entire study, the total cost was EUR 61,750. A one-year study, collecting and analyzing ca. 560–675 samples (2.5–3 per "assumed" number of bears, discussed later), would cost about EUR 66,710–77,750. The information costs are about the same for a 1- and 2-year project.

In order to carry out a helicopter-based estimate in an area similar to our study area and with a similar number of radio-marked bears (10 males and 20 adult females) and an annual loss rate of about 25%, one must capture and mark bears the year before the study starts in order to obtain a sufficient sample. This means that people must

be employed to locate bear tracks and bears for capture attempts (three persons working one month per year, costing a total of EUR 28,100 for 3 years, using cars and snowmobiles, costing EUR 20,500). It costs about EUR 2050 to mark a bear, including capture with helicopter, immobilization drugs and the radio transmitter, and 46 captures are needed, or totally EUR 94,300, in addition to 1.3 months work for each of two field assistants per year for 3 years, totally EUR 23,300. Finally, costs for the 2-year data collection project are EUR 7100 for receivers and other equipment for the helicopter, EUR 77,000 for helicopter time and other travel, and EUR 31,000 for wages for two assistants working 2 months each. Thus, the entire cost would be about EUR 281,300 or about 4.5 times greater than the genetic method. If the helicopter method were to be conducted for only 1 year, the cost would be about EUR 197,300, because the first start-up year would still be necessary. This is 2.5–3 times more than the estimate for the 1-year genetic study with 560–675 samples, respectively. We would like to stress that capturing and monitoring a large proportion of the population was not only done for estimating population sizes. It was also important in order to obtain basic information on brown bear biology (e.g. reproductive and survival rates, dispersal, home range sizes, habitat selection and behaviour).

Another aspect that should not be neglected when choosing the appropriate method to estimate population size of any animal is ethics (Bekoff and Jamieson, 1996). Traditional field methods involving captures of the animals imply potential dangers of injury or death to the animal (Arnemo et al., in press), although the mortality rate due to capture was very low in this study (0.4%). This is a concern especially for small or endangered populations. The non-invasive genetic method, besides being financially cheaper, also has several other advantages. First, the animals are not disturbed. Second, the genetic data obtained from the faecal analysis contain information that could be used for additional purposes not related to estimating population size, such as estimating population genetic parameters (i.e. genetic structure, gene flow or relatedness), although the number of markers required for this type of analysis might be higher than the number required for individual identification. Behavioural features (i.e. basic home ranges estimates) might also be assessed. Finally, the fact that local hunters voluntarily collected the faecal samples contributed to local acceptance of the results. In case of small populations, the genetic method would permit a genetic sampling of the entire population and follow the presence and geographical distribution of the different individuals from year to year.

4.4. Implications for conservation

In 1993, Swenson et al. (1995) estimated the density of bears in a 4100 km^2 area, entirely within our study area, to be 20/1000 km^2, using an airplane-based CMR method. We estimated densities of 24 and 20 bears/1000 km^2 in 2001 and 2002, respectively, using a similar helicopter CMR method (Table 1). Thus, the density estimates in our present study are very similar to that about 10 years ago, and were prob-

ably about 30 bears/km^2 in both periods, based on the results of the two genetic methods. Similar high densities have also been reported in other bear populations (McLoughlin et al., 2000). It is possible that the brown bear has reached a threshold density in our study area or that population growth has been stopped due to hunter harvest. Based partially on this study, the brown bear population in Sweden has been estimated to be 1600–2800 in 2004, with a population growth rate of about 4.7% annually (Kindberg et al., 2004). Therefore, the present management of the population has been successful and bears in Sweden can be considered as being in a good conservation status. This gives managers more flexibility in their decisions, including the setting of hunting quotas and the removal of problem bears, which are both important questions in management (Swenson et al., 2000).

5.   Recommendations for management

We recommend the MARK method based on non-invasive genetic data, when possible, for future population size estimation of brown bears. This method appeared to be more reliable than the other field methods, had relatively small confidence intervals and costs one-third to one-fifth as much as the helicopter-based CMR method, in addition to the other advantages described above. Another approach is to combine various types of data from different sources and scales through powerful modeling approaches to obtain indirect estimates of population size (Wiegand et al., 2003). Such models can prove to be very useful in the conservation of rare and elusive animals like the brown bear where limited data is available.

However, we would like to point out two main concerns about the genetic method. First, technical difficulties in the laboratory work, associated with low quality and low quantity DNA samples (Taberlet et al., 1999), should not be underestimated. Our own experience has shown that the DNA amplification success is unpredictable and depends on different factors, such as conservation conditions of feces in the field. For instance, the genotyping success rate seems higher for faecal samples collected in colder climates (P.Taberlet pers comm.). Therefore, we recommend pilot studies to be conducted for every project aiming to estimate population size from non-invasive data (Hedmark et al., 2004; Maudet et al., 2004). Secondly, the genetic method appeared to be sensitive to sampling intensity. It performed well in 2001, when 1.6 samples with usable DNA were collected per estimated bear, but less well in 2001, when the ratio was 0.7. We stress the importance of an adequate sampling. Future studies should aim at collecting 2.5–3 times the number of faecal samples as the "assumed" number of bears (considering that approximately 20–30% of the samples could not be genotyped). Depending on the estimates obtained after data analyses, the sampling effort could then be re-adjusted to obtain more reliable estimates.

Reliable population size and density estimates are important for successful management of any animal. We hope our study will contribute to improve future population size and density estimates especially of elusive animals.

Appendix 1

Survival statistics from the southern part of Sweden, 1996–2001			
Age	Males and females combined	Lower CI	Upper CI
0	0.531 (140[a])	0.489	0.574
1	0.804 (43[a])	0.725	0.882
a Bear years.			

Demographic data from the southern part of Sweden, 1996–2001			
	Mean	Lower CI	Upper CI
Proportion of females in oestrus	0.64	0.72	0.56
Reproductive rate (cubs/adult female/year)	1.42		

Appendix 2

Equation to calculate the total population size based on

(a) Females with cubs

$$N = F + M + CUBS + YEAR + TWO$$
$$F = \frac{FC}{PFC}$$
$$M = F$$
$$CUBS = REPR \times F$$
$$YEAR = CUBS \times Scubs$$
$$TWO = YEAR \times Syear$$

N – Estimated total population size
F – Total number of females $\geqslant$ 3 years
FC – Estimated number of females with cubs
PFC – (1 – Proportion females in oestrus)

M – Total number of males $\geqslant$ 3 years
$CUBS$ – Number of cubs of the year
$Scubs$ – Cub survival rate
$REPR$ – Reproductive rates among adult females
$YEAR$ – Number of yearlings
$Syear$ – Yearling survival rate
TWO – Number of 2 year olds

(b) Females in oestrus

$$N = F + M + CUBS + YEAR + TWO$$
$$F = \frac{FO}{PFO}$$
$$M = F$$
$$CUBS = REPR \times F$$
$$YEAR = CUBS \times Scubs$$
$$TWO = YEAR \times Syear$$

N – Estimated total population size
F – Total number of females $\geqslant$ 3 years
FO – Estimated number of females in oestrus
PFO – Proportion females in oestrus
M – Total number of males $\geqslant$ 3 years
$CUBS$ – Number of cubs of the year
$Scubs$ – Cub survival rate
$REPR$ – Reproductive rates among adult females
$YEAR$ – Number of yearlings
$Syear$ – Yearling survival rate
TWO – Number of 2 year olds

REFERENCES

Arnason, A.N., Schwarz, C.J., Gerhard, J.M., 1991. Estimating closed population size from sightings of marked and unmarked animals. J. Wildlife Manage. 55, 716–730.

Arnemo, J.M., Ahlqvist, P., Andersen, R., Berntsen, F., Ericsson, G., Odden, J. Brunberg, S., Segerström, P., Swenson, J.E., in press. Risk of capture-related mortality in large free-ranging mammals: experiences from Scandinavia. Wildlife Biol., in press.

Banks, S.C., Piggott, M.P., Hansen, B.D., Robinson, N.A., Taylor, A.C., 2002. Wombat coprogenetics: enumerating a common wombat population by microsatellite analysis of faecal DNA. Australian J. Zool. 50, 193–204.

Banks, S.C., Hoyle, S.D., Horsup, A., Sunnucks, P., Taylor, A.C., 2003. Demographic monitoring of an entire species (the northern hairy-nosed wombat, *Lasiorhinus krefftii*) by genetic analysis of non-invasively collected material. Conserv. Genetics 4, 101–107.

Bartmann, R.M., White, G.C., Carpenter, L.H., Garrott, R.A., 1987. Aerial mark-recapture estimates of confined mule deer in pinyon-juniper woodland. J. Wildlife Manage. 51, 41–46.

Bekoff, M., Jamieson, D., 1996. Ethics and the study of carnivores: doing science while respecting animals. In: Gittleman, J.L. (Ed.), Carnivore Behavior, Ecology, and Evolution, Volume 2. Cornell University Press, Ithaca, New York, pp. 15–45.

Bellemain, E., Taberlet, P., 2004. Improved non-invasive genotyping method: application to brown bear (*Ursus arctos*) faeces. Mol. Ecol. Notes 4, 519–522.

Bellemain, E., Swenson, J.E., Tallmon, D., Brunberg, S., Taberlet, P., 2005. Estimating population size of elusive animals using DNA from hunter-collected faeces: comparing four methods for brown bears. Conserv. Biol. 19 (1), 150–161.

Buckland, S.T., Anderson, D.R., Burnham, K.P., Laake, J.L., 1993. Distance sampling: estimating abundance of biological populations. Chapman and Hall, New York.

Castley, J.G., Knight, M.H., Mills, M.G.L., Thouless, C., 2002. Estimation of the lion (*Panthera leo*) population in the southwestern Kgalagadi Transfrontier Park using a capture–recapture survey. African Zool. 37, 27–34.

Dahle, B., Swenson, J.E., 2003a. Family breakup in brown bears: are young forced to leave? J. Mammal. 84 (2), 536–540.

Dahle, B., Swenson, J.E., 2003b. Home ranges in adult Scandinavian brown bears *Ursus arctos*: effect of mass, sex, reproductive status, population density and habitat type. J. Zool. 260, 329–335.

Eberhardt, L.L., Knight, R.R., 1996. How many grizzlies in Yellowstone? J. Wildlife Manage. 60, 416–421.

Eggert, L.S., Eggert, J.A., Woodruff, D.S., 2003. Estimating population sizes for elusive animals: the forest elephants of Kalum National Park, Ghana. Mol. Ecol. 12, 1389–1402.

Elgmork, K., Brekke, O., Selboe, R., 1976. Reliability of Observations from the Public of Tracks and Signs of the Brown Bear, *Ursus arctos* L. *Fauna*, vol. 29, first ed. Chapman & Hall, London, pp. 45–50 (In Norwegian with English summary).

Flagstad, O., Hedmark, E., landa, A., Broseth, H., Persson, J., Andersen, R., Segerstöm, P., Ellegren, H., 2004. Colonization history and non-invasive monitoring of a re-established wolverine population. Conserv. Biol. 18 (3), 676–688.

Greenwood, R.J., Sargeant, A.B., Johnson, D.H., 1985. Evaluation of mark-recapture for estimating striped skunk abundance. J. Wildlife Manage. 49, 332–340.

Hallet, J.G., O'Connell, M.A., Saunders, G.D., Seidensticker, J., 1991. Comparison of population estimators for medium-sized animals. J. Wildlife Manage. 55, 81–93.

Hedmark, E., Flagstad, O., Segerstrom, P., Persson, J., Landa, A., Ellegren, H., 2004. DNA-based individual and sex identification from wolverine (*Gulo gulo*) faeces and urine. Conserv. Genetics 5, 405–410.

Keating, K.A., Swartz, C.C., Haroldson, M.A., Moody, D., 2002. Estimating numbers of females with cubs-of-the-year in the Yellowstone grizzly bear population. Ursus 13, 16–174.

Kendall, K.C., Metzgar, L.H., Patterson, D.A., Steele, B.M., 1992. Power of sign surveys to monitor population trends. Ecol. Appl. 2, 422–430.

Kindberg, J., Swenson, J.E., Brunberg, S., Ericsson, G., 2004. Preliminär rapport on populations utveckling och storlek av brunbjörn i Sverige, 2004. Report from The Scandinavian Brown Bear Research Project to the Swedish Environmental Protection Agency, 10 pp.

Knight, R.R., Blanchard, B.M., Eberhardt, L.L., 1995. Appraising status of the Yellowstone grizzly bear population by counting females with cubs-of-the-year. Wildlife Soc. Bull. 23, 245–248.

Kohn, M.H., York, E.C., Kamradt, D.A., Haught, G., Sauvajot, R.M., Wayne, R.K., 1999. Estimating population size by genotyping faeces. Proc. Roy. Soc. London 266, 657–663.

Kolstad, M., Mysterud, I., Kvam, T., Sorensen, O.J., Wikan, S., 1986. Status of the brown bear in Norway; distribution and population 1978–1982. Biol. Conserv. 38, 79–99.

Krebs, C.J., 1999. Ecological Methodology, second ed. Benjamin/Cummings, Menlo Park, CA.

Kristoffersen, S., 2002. Restricting daily movements as a counterstrategy against sexually selective infanticide in brown bears (*Ursus arctos*). Cand. Scient. Thesis, University of Oslo.

Linnell, J.D.C., Swenson, J., Landa, A., Kvam, T., 1998. Methods for monitoring European large carnivores – A worldwide review of relevant experience. Oppdragsmelding 549, Norwegian Institute for Nature Research, Trondheim (In Norwegian).

Mattson, D.J., 1997. Sustainable grizzly bear mortality calculated from counts of females with cubs-of-the-year: an evaluation. Biol. Conserv. 81, 103–111.

Maudet, C., Luikart, G., Dubray, D., Von Hardenberg, A., Taberlet, P., 2004. Low genotyping error rates in wild ungulate faeces sampled in winter. Mol. Ecol. Notes 4, 772–775.

McCullough, D.R., Hirth, D.H., 1988. Evaluation of the Petersen–Lincoln estimator for a white-tailed deer population. J. Wildlife Manage. 52, 534–544.

McLoughlin, P.D., Ferguson, S.H., Messier, F., 2000. Intraspecific variation in home range overlap with habitat quality: a comparison among brown bear populations. Evol. Ecol. 14, 39–60.

Miller, S.D., White, G.C., Sellers, R.A., Reynolds, H.V., Schoen, J.W., Titus, K., Barnes Jr., V.G., Smith, R.B., Nelson, R.R., Ballard, W.B., Schwartz, C.C., 1997. Brown and black bear density estimation in Alaska using radiotelemetry and replicated mark-resight techniques. Wildlife Monographs 133, 1–55.

Miller, S.D., Becker, E.F., Ballard, W.B., 1987. Black and Brown bear density estimates using modified capture–recapture techniques in Alaska. Int. Conference Bear Res. Manage. 7, 23–35.

Minta, S., Mangel, M., 1989. A simple population estimate based on simulation for capture–recapture and capture-resight data. Ecology 70, 1738–1751.

Naturårdsverket, 2003. Åtgärdsprogram för bevarande av björn (*Ursus artos*). Åtgärdsprogram No. 20, Swedish Environmental Protection Agency, Stockholm (In Swedish).

Neal, A.K., White, G.C., Gill, R.B., Reed, D.F., Olterman, J.H., 1993. Evaluation of mark-resight model assumptions for estimating mountain sheep numbers. J. Wildlife Manage. 57, 436–450.

Paetkau, D., 2003. An empirical exploration of data quality in DNA-based population inventories. Mol. Ecol. 12, 1375–1387.

Paetkau, D., Strobeck, C., 1994. Microsatellite analysis of genetic variation in black bear populations. Mol. Ecol. 3, 489–495.

Piggott, M., Bellemain, E., Taberlet, P., Taylor, A.C., 2004. A multiplex pre-amplification method that significantly improves microsatellite amplification and error rates for fecal DNA in limiting conditions. Conserv. Genetics 5, 417–420.

Sandegren, F., Swenson, J. 1997. Björnen—viltet, ekologin och människan. Svenska Jägareförbundet, Stockholm, Sweden, 70 pp. (In Swedish).

Schwartz, C.J., Seber, G.A.F., 1999. Estimating animal abundance: review III. Statistical Sci. 14, 427–456.

Seber, G.A.F., 1982. The Estimation of Animal Abundance, second ed. Macmillan, New York.

Selander, S., Fries, C., 1943. 1942 års björnutredning. Sveriges Natur 1, 1–10 (In Swedish).

Smallwood, K.S., Schonewald, C., 1998. Study design and interpretation of mammalian carnivore density estimates. Oecologia 113, 474–491.

Smith, K.L., Alberts, S.C., Bayes, M.K., Bruford, M.W., Altmann, J., Ober, C., 2000. Cross-species amplification, non-invasive genotyping, and non-mendelian inheritance of human STRPs in savannah baboons. Am. J. Primatol. 51, 219–227.

Swenson, J.E., Gerstl, N., Dahle, B., Zedrosser, A., 2000 Action plan for the conservation of the brown bear (*Ursus arctos*) in Europe. Council of Europe, Report T-PVS, p. 24.

Swenson, J.E., Sandegren, F., Söderberg, A., 1998. Geographic expansion of an increasing brown bear population: evidence for presaturation dispersal. J. Anim. Ecol. 67, 819–826.

Swenson, J.E., Sandegren, F., Bjärvall, A., Söderberg, A,, Wabakken, P., Franzen, R., 1994. Size, trend, distribution and conservation of the brown bear (*Ursus arctos*) population in Sweden. Biol. Conserv. 70, 9–17.

Swenson, J.E., Wabakken, P., Sandegren, F., Bjärvall, A., Franzen, R., Söderberg, A., 1995. The near extinction and recovery of

brown bears in Scandinavia in relation to the bear management policies of Norway and Sweden. Wildlife Biol. 1, 11–25.

Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L.P., Bouvet, J., 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucl. Acids Res. 24, 3189–3194.

Taberlet, P., Waits, L.P., Luikart, G., 1999. Non-invasive genetic sampling : look before you leap. Trends Ecol. Evol. 14, 323–327.

Wabakken, P., Maartman, E., 1994. Sluttrapport for bjorn-sauprosjektet I Hedmark 1990–1993. Forskningsrapport 058. Norwegian Institute for Nature Research, Trondheim (In Norwegian).

Waits, L., Taberlet, P., Swenson, J.E., Sandegren, F., 2000. Nuclear DNA microsatellite analysis of genetic diversity and gene flow in the Scandinavian brown bear (*Ursus arctos*). Mol. Ecol. 9, 421–431.

Wesley, M., Hochachka, K.M., Doyle, F., Krebs, C.J., 2000. Monitoring vertebrate populations using observational data. Can. J. Zool. 78, 521–529.

White, G.C., Anderson, D.R., Burnam, K.P., Otis, D.L., 1982. Capture–recapture and Removal Methods for Sampling Closed Populations. Los Alamos National Laboratory, Los Alamos, NM.

White, G.C., 1996a. Program NOREMARK Software Reference Manual. Department of Fisheries and Wildlife, Colorado State University, Fort Collins.

White, G.C., 1996b. NOREMARK: population estimation from mark-resight surveys. Wildlife Soc. Bull. 24, 50–52.

White, G.C., Burnham, K.P., 1999. Program MARK: survival estimation from populations of marked individuals. Bird Study Suppl. 46, 120–138.

Wiegand, T., Naves, J., Stepha, T., Fernandez, A., 1998. Assessing the risk for extinction for the brown bear (*Ursus arctos*) in the Corillera Cantabrica, Spain. Ecol. Monographs 68, 539–570.

Wiegand, T., Jeltsch, F., Hanski, I., Grimm, V., 2003. Using pattern-oriented modeling for revealing hidden information: a key for reconciling ecological theory and application. Oikos 100, 209–222.

Wilson, G.J., Frantz, A.C., Pope, L.C., et al, 2003. Estimation of badger abundance using faecal DNA typing. J. Appl. Ecol. 40, 658–666.