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Is Non-invasive Genetic Population Estimation via Faeces Sampling Feasible for Abundant Mammals with Low Defecation Rates? A Pilot Study on Free Ranging Wild Boar (*Sus scrofa)* in South-West Germany

Cornelia EBERT[ad*] – Karolina KOLODZIEJ[b] – Tim Frederic SCHIKORA[c] – Holger K. SCHULZ[b] – Ulf HOHMANN[a]

[a] Research Institute for Forest Ecology and Forestry, Trippstadt, Germany
[b] Institute for Environmental Sciences, University of Koblenz-Landau, Germany
[c] Institute for Ecology, Evolution & Diversity, Goethe-University of Frankfurt, Germany
[d] Department of Wildlife Ecology and Management, University of Freiburg, Germany

Abstract – Wild boar is a widespread and abundant species for which until now reliable and accurate population estimates are still lacking. In this study, a method based on non-invasive genetic sampling applied in a mark-recapture framework is tested. Faeces collected along line transects serve as DNA source. Aim of the study was to evaluate efficiency and practicability of the sampling design and to assess if a sample size sufficient for reliable population estimation can be achieved. In a 12-day sampling trial which was conducted in winter 2006 and covered approx. 25 km², 4 persons collected 141 fresh wild boar faeces originating from 74 different individuals. This sample size was below those recommended for non-invasive mark-recapture studies. Population estimates calculated using program CAPTURE strongly differed between models. Even though the non-invasive approach worked in principle for wild boar, further research will have to focus on increasing sample size while keeping cost and effort acceptable for a large scale application of the method.

mark-recapture / genotyping / transect / sample size/ population density

Kivonat – Alkalmas-e a hullatékgyűjtéses mintavétel a gyakori emlősök non-invazív genetikai populáció becslésére, alacsony ürítési ráta esetén? Esettanulmány szabad területen élő vaddisznókon (*Sus scrofa*) Délnyugat-Németországban. A vaddisznó egy széles körben elterjedt és gyakori faj, amelynek megbízható és pontos genetikai populáció becslése ez idáig nem történt meg. Ebben a vizsgálatban a módszer a non-invazív genetikai mintavételen alapult, amelyet az alkalmazott jelölés-visszafogás módszer keretében valósítottunk meg. A vonaltranszekt mentén gyűjtött hullatékok nyújtották a DNS forrást. Kutatásunk célja volt, hogy megbecsüljük a mintavételi módszer kivitelezésének hatékonyságát és használhatóságát, és megállapítsuk azt a mintaméretet, amely elegendő a megbízható populációbecsléshez, és megvalósítható. A 2006 telén körülbelül 25 km²-en végzett 12 napos mintagyűjtés alatt, 4 fő 141 friss vaddisznó-hullatékot gyűjtött, 74 különböző egyedtől. Ez a mintaszám nem érte el a non-invazív jelölés-visszafogás módszerhez ajánlott elemszámot. A CAPTURE programmal végzett populációbecslés határozottan eltért a modellek között. Annak ellenére, hogy a non-invazív megközelítés alapjaiban működött a

* Corresponding author: cebert@gmx.de; Hauptstraße 16, D-67705 Trippstadt, Germany

vaddisznó esetében, a további kutatásokban az elemszám növelésére kell fókuszálnunk úgy, hogy a költségeket ne növeljük, és a ráfordított munka elfogadható legyen a módszer alkalmazásánál.

befogás / genotipizálás / transzekt / mintanagyság/ populáció sűrűség

1 INTRODUCTION

Population estimation is an important task for the management of wild boar, in particular with respect to the epidemiological role wild boar play in the transmission of the classical swine fever (Artois et al. 2002) or in order to evaluate efficiency of hunting measures. In research for methods that enable to obtain reliable data and are less biased than most traditional approaches (e.g. hunting bag analysis or traditional mark-recapture), strategies based on non-invasive genetic sampling yield promising results for several species (Piggott – Taylor 2003). The tissue sources most commonly used for population estimation in mammals are hair and faeces. Population estimation via hair sampling has been applied for several different species, e.g. grizzly *Ursus arctos* and black bears *U. americanus* (Mowat et al. 2005) and badgers *Meles meles* (Scheppers et al. 2007). Faeces have served as DNA source e.g. in estimation of coyote *Canis latrans* (Kohn et al. 1999), African elephant *Loxodonta africana* (Eggert et al. 2003) and lesser horseshoe bat *Rhinolophus hipposiderus* (Puechmaille – Petit 2007) populations. After individual identification of samples via genotyping, a modified capture-mark-recapture approach can be applied for population estimation (Woods et al. 1999).

For wild boar, the suitability of both hair and faeces as DNA sources has been tested (Fickel – Hohmann 2006). For wild boar like for other species hair is more favourable compared to faeces in terms of DNA quality and quantity (Franz et al. 2004, Fickel – Hohmann 2006, Regnaut et al. 2006). However, a pilot study conducted in the field revealed that hair sampling at baited stations is not practicable for reliable population estimation (Ebert et al. submitted): behaviour of wild boar at the stations differed strongly dependent on individual age and group status, resulting in heterogeneous individual sampling probabilities. As an alternative, we collected wild boar faeces along transects in a forested area in southwestern Germany. Our aim was to develop a reliable, representative and cost-effective sampling strategy for non-invasive population estimation. In this respect, obtaining a sufficient sample size is an important factor. For non-invasive genetic population estimation, several authors recommend collecting 2 to 3 times as many samples as animals are assumed to be present in the sampled population (Miller et al. 2005, Solberg et al. 2006). This recommendation is based partly on the fact that a certain proportion of the samples will have to be discarded from genetical analysis due to low DNA quality or quantity (Puechmaille – Petit 2007). In general, when intending to apply mark-recapture methods, the best way to obtain estimates with low bias and good precision is to ensure high capture probabilities and a high rate of recaptures (White et al. 1982). This necessitates an intensive sampling. On the other hand, a method has to be kept feasible. Thus, we aimed at evaluating the practicability and efficiency of a faeces sampling design based on line transects. Compared to other ungulates, wild boar have a low defecation rate (Briedermann 1990, Stubbe et al. 1997). Consequently, obtaining a sufficiently large sample is a crucial point in this context. Furthermore, wild boar are a widespread and abundant species, the faeces of which will distribute over wide areas. This exacerbates the difficulty of obtaining a sufficient sample size. Furthermore, it may limit the scope of non-invasive methods in terms of cost and effort for wild boar compared to rare and/ or endangered species.

We conducted our sampling trial in winter in order to keep loss of samples due to degradation and insects as low as possible. Furthermore, sampling during low ambient temperatures has been shown to increase genotyping success e.g. in wolves Canis lupus (Luccini et al. 2002), wolverines Gulo gulo (Hedmark et al. 2004), mouflon (Ovis musimon) and alpine ibex Capra ibex (Maudet et al. 2004). Furthermore, by repeating the same transect routes as

accurate as possible for every sampling occasion, we intended to maximize the possibility of collecting fresh faeces (i.e. less than 48 hours old), which has been shown to increase genotyping success (see e.g. Arrendal et al. 2007, Murphy et al. 2007, Santini et al. 2007).

2 METHODS

2.1 Study area

Faeces sampling was carried out in a site of 2500 ha situated in the Palatinate Forest in southwestern Germany (49°12'N, 7°45' E). Elevation ranges mostly from 250 to 450 m a.s.l. with a minimum of 210 m and a maximum of 609 m. Hills and valleys are orientated mainly from northeast to southwest. The predominant native plant community is beech forest (Luzulo-Fagetum). The area is covered with forest to approximately 90% (44% *Fagus sylvatica*, 26% *Pinus* sp., 10% *Picea abies*, 12% *Quercus petraea* and *Quercus robur*; Reis 2006). Several small settlements with surrounding open areas lie at the periphery of the study area. Annual average temperature is 8-9°C (Weiß 1993), annual precipitation approximates 600 – 1000 mm.

Three ungulate species occur in the Palatinate Forest: red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*). The annual harvest of wild boar in the state-hunting areas between 1999 and 2006 averages 2.7 individuals per km² (Range: 1.14 to 5.23 individuals per km² and year; Reis 2006). The hunting bag in the study year was comparably low: 1.6 wild boar per km².

2.2 Faeces sampling and genotyping

Sampling was carried out between November 27th and December 12th 2006. Wild boar faeces were collected along 16 transects of approx. 7 to 8 km length each *(Figure 1)*. Transects were installed parallel to each other in direction from north to south (overall length: 104 km). Trails, small roads or streams were crossed, if necessary, but it was avoided to conduct transects along trails or roads, in order to prevent potential bias of sampling results. The parallel N-S transect design was chosen with the aim to cover the study area as representative as possible by including all occurring habitat types and altitudinal levels. Four persons each walked two transects per day. Thus, all 16 transects were searched within 48 hours. The total of 16 sampling days was divided into 2 blocks of 8 days with a break of 4 days in between. Thus, each transect was searched 8 times in total within a period of approx. 3 weeks. In order to ensure that the same transect routes were searched in every repetition, transects were marked using spray paint on trees. The transect width which could be effectively searched for wild boar faeces by a walking person was approximately 3 m.

Whole faeces were stored frozen (-19ºC) in sealed plastic bags until analysis. Genotyping of samples was carried out in the laboratories of the University of Koblenz-Landau, Germany, based on 4 microsatellite loci and one Y-linked sex marker (Kolodziej et al. 2008). In order to obtain reliable consensus genotypes, all homozygous loci were repeated 10 times, whereas for heterozygous loci, 3 successful repeats were carried out.

Based on the genotyping results, population sizes were calculated using program CAPTURE (White et al. 1978). For later comparison, we chose 5 different models from the program:

- the null model (M0) which assumes equal sampling probability for all individuals in the population, no behavioural response to sampling and no variation over time
- Mt assuming a variation in sampling probability over time
- Mh Jackknife (Mh J) and Mh Chao (Mh C) assuming individual heterogeneity of sampling probabilities
- Mth Chao (Mth C) assuming sampling probability to vary over time and due to individual heterogeneity

The two Chao models have been developed especially for use with small sample sizes (Chao 1989).

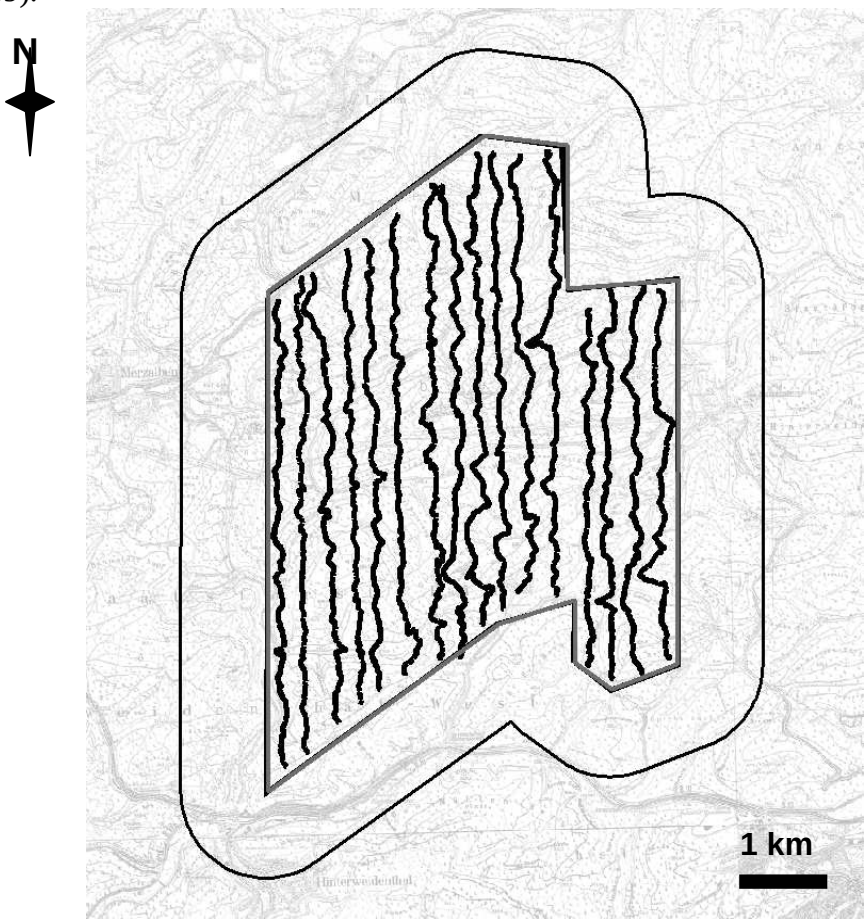

Figure 1. Transect design in the study area (25 km²) and buffer with the width of a mean monthly wild boar home range radius marking the effective sampling area (52 km²)

Additionally, we incorporated the model selection process of program CAPTURE which suggests an 'appropriate' model following the results of program-inherent goodness-of-fit tests.

Because in our study area the population can not be assumed to be closed, population densities have been calculated with a buffer of 1000 m around the study area, which corresponds to the radius of an average monthly 95% MCP-home range of wild boar radiotracked in the study area (Ebert et al. 2007). Thus, the area used for density calculation corresponds to 5200 ha.

3 RESULTS

3.1 Faeces sample collection

In 12 sampling days, 141 wild boar faeces were collected (*Figure 2*). To obtain these samples, a total of 622 km of transects were covered. The sampling was carried out by four persons; total time expended was 335 man-hours. This corresponds to 0.23 samples per km of transect and 0.42 samples per man-hour, respectively. The number of wild boar sampled per day varied considerably in both sampling blocks (day 1 to day 6 and day 7 to day 12 respectively). In both cases, it showed a decline from the first day to the last day of each block (*Figure 1*).

Of the 141 samples, 89 (63%) were genotyped successfully, representing 74 individual animals. The frequencies with which wild boar were sampled 1, 2, 3, 4 and 5 times were 66, 4, 3, 0, 1, respectively. This corresponds to 14 resampling events altogether. Of the 74 individuals, 48 were female and 26 were male (sex ratio male : female 1 : 1.84).

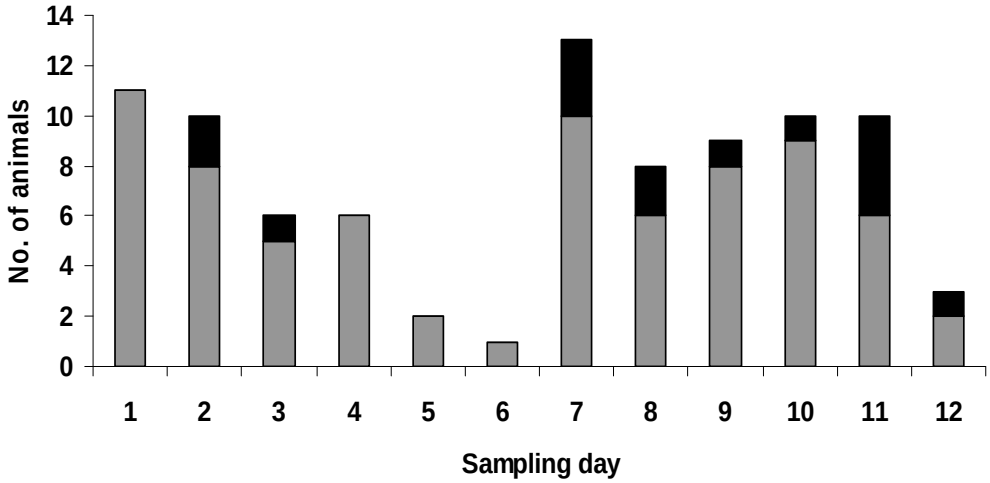

Figure 2. Number of wild boar sampled per day.
The number of animals sampled first time is given in grey, recaptures are given in black.

3.2 Population estimation

Model selection routine in program CAPTURE suggested a time specific variation in the sampling probabilities ($Chi^2 = 39.335$, df = 11, $p < 0.001$) as well as the possibility of individual heterogeneity ($Chi^2 = 22.430$, df = 11, $p = 0.021$). CAPTURE suggested model Mt as the appropriate estimator. The different models give estimated sampling probabilities of about 0.02 (2%) per sampling day. The point estimates and confidence intervals as well as the population density vary between the different models (*Table 1*). In order to evaluate the degree of coverage, we calculated the ratio sample size/ estimated population size to enable comparison with the recommended sample sizes (see introduction). Averaging over the different models' results, we obtained in mean 0.44 samples per wild boar assumed to be in the sampled population *(Table 1).*

Table 1. Population estimates and population densities derived from wild boar faeces samples using different models in program CAPTURE (see text for descriptions of the models). Population densities (wild boar per km²) were calculated based on an effective sampling area of 52 km². The mean sampling probabilities are estimates generated in program CAPTURE.

Estimation model	M0	Mt	Mh J	Mh C	Mth C
Population size N (95% CI)	225 (153 – 364)	221 (151 – 355)	308 (248 – 391)	619 (270 – 1587)	523 (270 – 1106)
Population density (95% CI)	4.3 (2.9 – 7.0)	4.3 (2.9 – 6.8)	5.9 (4.8 – 7.5)	11.9 (5.2 – 30.5)	10.0 (5.2 – 21.3)
Mean sampling probability	0.032	0.034	0.024	0.011	0.014
Ratio collected faeces/ estimated N	0.63	0.64	0.46	0.23	0.27

4 DISCUSSION

4.1 Sample size considerations

Considering the recommendations and theoretical requirements of traditional mark-recapture methods, the sample size achieved in our faeces sampling trial seems small (see e.g. Otis et al. 1978, White et al. 1982). This also holds true with respect to sample size recommendations based on the experiences of other non-invasive genetic population studies (Puechmaille – Petit 2007, Solberg et al. 2006): In order to achieve the aim of collecting 2 to 3 times as many samples as the assumed number of wild boar in our study area - even if we take the lowest of our estimates (Model Mt) as a measure - the desired sample size in our case would have been 442 to 663 faeces samples. Consequently, the sampling probabilities estimated in program CAPTURE for our data are low. While Otis et al. (1978) state that 'capture' probabilities have to be at least 0.1 for each capture occasion to obtain reliable results, in our study the estimated probabilities ranged model-dependent from 0.011 to 0.034. Thus, even though the faeces sampling procedure worked in principle for wild boar, the number of collected faeces will have to be increased considerably in the future. Consequently, the number of samples collected is only 0.23 to 0.64 times the estimated number of wild boar, dependent on which model is chosen. One reason for the low sample size may be the rather low defecation rate of wild boar compared to other ungulates. While the mean number of defecations per 24 hours in wild boar averages 4.5 (Briedermann 1990), the rate in red deer (*Cervus elaphus*) is 19 and in roe deer (*Capreolus capreolus*) 14, respectively (Tottewitz et al. 1998). A survey of red deer faeces carried out in our study area in spring 2009 yielded a sampling success of 1.6 samples per km of transect (M. Rahlfs, pers. comm.) – this is almost seven times the density of wild boar faeces, even though wild boar are assumed to be more abundant in this area than red deer. However, faeces sampling also has been carried out for some carnivores with defecation rates comparable to those of wild boar, e.g. brown bears *Ursus arctos* and Iberian lynx *Lynx pardinus* (Bellemain et al. 2005, Palomares et al. 2002). But in species like e.g. lynx or under colder or drier climatic conditions, faeces can be suitable for analysis for longer time compared to wild boar in our study area. The condition that even older faeces have to be successfully analyzable can be crucial for the practicability of the method especially when applied to rare and elusive species (Palomares et al. 2002). For wild boar faeces, DNA quality seems to decrease considerably from 48 h after defecation, with some variations depending on weather conditions (S. Eckert, unpublished data). Similar patterns have been shown for several other species (Fernando et al. 2000, Piggot 2004). Thus, frequent searching of transects is important for obtaining samples as fresh as possible. For this reason, we searched all transects every second day in our study, thus ensuring that the age of the majority of samples is less than 48 hours.

The most obvious method to increase sample size is to raise sampling effort. However, this can affect the feasibility of a method dependent on the facilities available. A promising approach for more effective faeces collection, which has already been applied successfully e.g. to grizzly bears (*Ursus arctos*), is the search with trained dogs (Wasser et al. 2004, Long et al. 2007). Dogs have been shown to reach significantly higher faeces detection rates compared to humans (Smith et al. 2005). However in wild boar, depending on area and population, the prevalence of Aujeszky's disease – which is lethal for dogs like for most carnivores (Bastian et al. 1999, Müller et al. 1998) can be more or less strong. This holds the risk of infection for detection dogs, since Aujeszky-Virus has also proven to be present in wild boar faeces (C. Adlhoch, pers. communication). Thus, this sampling method does not seem to be feasible for wild boar. The necessary increase in sample size should therefore be realised by increasing sampling intensity (longer period, more observers, more/ longer transects) or by a change of sampling strategy (e.g. by combination with hunting bag or some other kind of additional sampling).

4.2 Population estimation

The population estimates and confidence intervals derived from the capture histories of the 74 wild boar show considerable variation dependent on the applied models. Models M0 and Mt show very similar results. Resulting from the differences between the trial days in the number of wild boar sampled, CAPTURE model selection suggested model Mt as appropriate. But considering the biology and behaviour of wild boar and also the results of the majority of non-invasive studies, we would expect a certain heterogeneity in the sampling probabilities (Knapp et al. 2002). The Jackknife Mh model, which is known to perform well with large samples (Burnham – Overton 1978, Chao 1987), yielded a higher estimate compared to the models not incorporating heterogeneity. The two Chao models (Mh Chao and Mth Chao), which both incorporate heterogeneity and which are said to be especially suited for small samples like ours, show very high estimates and much larger confidence intervals compared to the others. The densities obtained from our data with those two models lie in the range of the highest wild boar densities reported by Hebeisen et al. (2007). Compared to Mh Jackknife, Mh Chao gives a population estimate twice as high.

The question which one of the estimates is closest to the real population size is difficult to answer. It has been shown previously, that the model selection procedure in program CAPTURE has low power in many cases, especially at low sample sizes (Menkens et al. 1988, McKelvey – Pearson 2001). Furthermore, part of the model selection tests failed with our data because the expected values were too small. As a consequence, we would not consider the suggested appropriate model Mt as the most suitable. Menkens et al. (1988) state that for very small data sets the Lincoln-Petersen estimator may provide more reasonable results as the more complex CAPTURE models. When applying the Lincoln-Petersen estimator (in its bias-corrected form; Chapman 1951) to our data set by setting day 1 to 6 as the ‘capture’ and day 7 to 12 as the ‘recapture’, we obtain a population estimate of 265 wild boar. This estimate lies in between those of the models M0, Mt and Mh Jackknife. Considering the different results while taking into account our very small sample and the statements of Menkens et al. (1988), the real population size may be best reflected by the less complex models. These seem quite reasonable for our study area and the study year: When comparing with densities estimated during previous studies in other parts of Europe (as reviewed in Hebeisen et al. 2007), the densities in habitats similar to our study area were comparable or even lower. Considerably higher densities were mostly reported from habitats with more favourable conditions e.g. due to agricultural crops as food sources. Besides the fact that our study area is a rather poor habitat without agricultural areas, the hunting bag in the study year was very small compared to the years before (even though hunting effort did not change between the years), indicating that the population in 2006 was low even for this area. However, until now the possibility of a biased estimation due to edge effects or due to genotyping errors can not be ruled out and requires further investigations (see Kolodziej et al. 2008).

The sex ratio of the genotypes derived from the faeces samples could either represent the real ratio in the population or be an artefact due to the small sample size. Considering the sampling design, we do not believe the detection probability to vary strongly between the two sexes. In the year of our study, 83 wild boar have been harvested in the study area. The hunting bag of the drive hunts in winter 2006/2007 showed a similarly female-biased sex ratio (male : female 1 : 1.53 in the hunting bag compared to 1 : 1.84 in the faeces samples [Landesforsten Rhineland-Palatinate, pers. comm.]) in the study year compared to our faeces samples. In general, a hunting bag may not represent an unbiased sample of a population. However, in drive hunts harvesting of wild boar is much less selective compared to single hunt, and thus we assume the drive hunt sex ratio to be nearer to the real ratio in the population. Thus, the drive hunt sex ratio supports the idea that the detection of more females than males in our faeces sampling might reflect reality and not be a consequence of the small sample size.

4.3 Cost effectiveness

The costs for personnel and transport during the field work (4 persons working on 12 sampling days plus processing of the field data) amounted to 8,000 Euros (approx. 11,300 US$). Analysis of faeces samples in the lab (1 person working 2 months and costs for extraction kits, PCRs and sequencing) cost approx. 70 Euros per sample (99 US$). Thus, the costs for the analyses of 141 samples amounted to approx. 9,690 Euros (13,710 US$). Total costs of the sampling trial and genotyping thus were approx. 17,690 Euros (25,000 US$), of which 45% represent field work and 55% are laboratory costs.

Comparing this to other studies, our costs and effort, but also our yield (in form of samples) is low: The costs for a one-year study on brown bears (*Ursus arctos*) carried out by Solberg et al. (2006) amounted to 66,700 to 77,700 Euros (95,130 to 110,800 US$). However, in this study a total of almost 700 samples were collected and analysed in two years. In a second bear study, Wasser et al. (2004) used 8 persons and 4 trained dogs to collect bear faeces. They collected 880 grizzly and black bear (*Ursus arctos, U. americanus*) faeces samples in two sampling trials over two years. For the first sampling trial, a minimum of 250 km of transects were searched, the minimum transect length for the second trial was 600 km. Wasser et al. (2004) report costs of about 500 US$ per sample (of these, 44% attributed to personnel, 9% to field transport, 42% to DNA analyses and 5% to hormone analyses). Total costs for their first trial (400 samples) therefore amounted to approx. 200,000 US$ and for their second trial (480 samples) to approx. 220,000 US$.

Compared to our study, both bear studies worked on a much larger spatial scale (7328 km² and 5200 km²). Needless to mention that the abundance of wild boar is much higher and their movement behaviour is considerably smaller scaled compared to brown bears and black bears. The estimated densities of bears range from 0.021 bears per km² (Solberg et al. 2006) to 0.037 bears per km² (Wasser et al. 2004). Thus, even our lowest estimated densities (4.3 wild boar per km²) are two orders of magnitude higher compared to the estimated bear densities. In terms of effectivity and population coverage, the two bear studies yield considerably higher values: Solberg et al. (2006) collected 2.26 and 1.22 times as many samples in their two study years as the estimated number of bears, and Wasser et al. (2004) even obtained 17.14 times as many samples as they estimated bears in their population under study. In contrast to this, we will have to increase the wild boar sample size at least threefold in order to reach the ratio recommended by Miller et al. (2005) and Solberg et al. (2006).

We found no other studies which give an account of their cost and effort, so that material for comparison is scarce. But in relation to the two studies cited above, it becomes apparent that non-invasive population estimation is carried out in a much larger dimension in terms of cost and effort. However, it may be questionable if the same dimension of cost and effort is acceptable for a widespread and abundant (and not endangered) species like the wild boar, especially when application on a larger scale is desired.

4.4 Conclusions

The basic method of non-invasive population estimation via faeces sampling seems to work for wild boar. However, several problems remain to be solved before it will be possible to obtain unbiased and accurate estimates with the approach presented here. First of all, the population estimates presented in this paper depend on reliable lab analyses. With faecal samples from wild boar, accurate genotyping turned out to be particularly challenging. At the time of printing, reliability of the lab results had yet to be confirmed. Thus, presented estimates must be regarded as preliminary. Furthermore, the sample size will have to be increased considerably. Also, additional studies are needed in order to

assess if there are sources of bias which until now remain undetected. For example, the female-biased sex ratio we found in our faeces sample genotypes should be verified in order to evaluate if there exists a sex-related heterogeneity in sampling probability.

For wild boar management and to control the spread of the classical swine fever, reliable population estimates are highly desirable. However, if the method presented here is to be applied on a larger scale, a serious concern which deserves further research will be to obtain a sufficient sample size while keeping the cost and effort acceptable.

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REFERENCES

ARTOIS, M. – DEPNER, K.R. – GUBERTI, V. – HARS, J. – ROSSI, S. – RUTILI, D. (2002): Classical swine fever (hog cholera) in wild boar in Europe. Rev. Sci. tech. Off. int. Epiz. 21: 287- 303

ARRENDAL, J. – VILÀ, C. – BJÖRKLUND, M. (2007): Reliability of noninvasive genetic census of otters compared to field censuses. Conserv. Gen. 8: 1097-1107

BASTIAN, S. – BUFFEREAU, J.P. – LE DREAN, E. – BIND, J.L. – MÜLLER, T. – TOMA, B. (1999): La Maladie d'Aujeszky en France.- Epidémiologie et santé animale 38, 109-114 [in french]

BELLEMAIN, E. – SWENSON, J.E. – TALLMON, D. – BRUNBERG, S. – TABERLET, P. (2005): Estimating population size of elusive animals with DNA from hunter-collected feces: four methods for brown bears. Conserv. Biol. 19, 150-161

BURNHAM, K.P. – OVERTON, W.S. (1979): Robust estimation of population size when capture probabilities vary among animals. Ecology 60, 927-936

CHAO, A. (1987): Estimating the population size for capture-recapture data with unequal catchability. Biometrics 43, 783-791

Ebert, C. – BERGER, K. – HUCKSCHLAG, D. – NIKOLOV, I. – SCHIKORA, T. – SCHULZ, H.K. – HOHMANN, U. (2007): Kann man Wildschweine zählen? Eine Unersuchung über die nicht-invasive Gewinnung von Gewebeproben ur Verwendung bei der Bestandesschätzung von Wildschweinen. In DEGENHARDT, A. – WUNN, U. (editors), Proceedings of the „Sektion forstliche Biometrie und Informatik", DVFFA „Die grüne Reihe" Vol. 18, 2007, pp. 63-72 [in german]

EBERT, C. – HUCKSCHLAG, D. – SCHULZ, H.K. – HOHMANN, U. (submitted): Can hair traps sample wild boar (*Sus scrofa*) randomly fort he purpose of wild boar population estimation?

EGGERT, L.S. – EGGERT, J.A. – WOODRUFF, D.S. (2003): Estimating population size for elusive animals: the forest elephants of Kakum National Park, Ghana. Mol. Ecol. 12: 1389-1402

FERNANDO, P.J. – PFRENDER, M.E. – ENCALADA, S.E. – LANDE, R. (2000): Mitochondrial DNA variation, phylogeography and population structure of the Asian elephant. Heredity 84, 362-372

FICKEL, J. – HOHMANN, U. (2006): A methodological approach for non-invasive sampling for population size estimates in wild boars (Sus scrofa). Eur. J. Wildl. Res. 52: 28-33

FRANTZ, A.C. – POPE, L.C. – CARPENTER, P.J. – ROPER, T.J. – WILSON, G.J. – DELAHAY, R.J. (2003): Reliable microsatellite genotyping of the Eurasian badger (Meles meles) using faecal DNA. Mol. Ecol. 12: 1649-1661

HEBEISEN, C. – FATTEBERT, J. – BAUBET, E. – FISCHER, C. (2008): Estimating wild boar (Sus scrofa) abundance and density using capture-resights in Canton of Geneva, Switzerland.- Eur. J. Wildl. Res. 54: 391-401

HEDMARK, E. – FLAGSTAD, O. – SEGERSTRÖM, P. – PERSSON, J. – LANDA, A. – ELLEGREN, H. (2004): DNA-based individual and sex identification from wolverine (*Gulo gulo*) faeces and urine. Conserv. Gen. 5, 405 – 410

LONG, R.A. – DONOVAN, T.M. – MACKAY, P. – ZIELINSKY, W.J. – BUZAS, J.F. (2007): Comparing scat detection dogs, cameras, and hair snares for surveying carnivores.- J. Wildl. Manage. 71, 2018-2025

KNAPP, S.M. – CRAIG, B.A. – WAITS, L.P. (2009): Incorporating genotyping error into non-invasove DNA-based mark-recapture population estimates. J. Wildl. Manage. 73, 589-604

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. R. Soc. (Lond.) 266: 657-663

KOLODZIEJ, K. – SCHULZ, H.K. – EBERT, C. – HOHMANN, U. – HUCKSCHLAG, D. – SCHULZ, R. (2008): DNA-based analysis of scats for use in wild boar (*Sus scrofa*) population size estimation – evaluating a lab method. Abstract of the 7th Int. Symposium on Wild Boar and Sub-order Suiformes, Sopron, Hungary

LUCCHINI, V. – FABBRI, E. – MARUCCO, F. – RICCI, S. – BOITANI, L. – RANDI, E. (2002): Non-invasive molecular tracking of colonizing wolf (Canis lupus) packs in the western Italian Alps. Mol. Ecol. 11: 857-868

MAUDET, C. – LUIKART, G. – DUBRAY, D. – VON HARDENBERG, A. – TABERLET, P. (2004): Low genotyping error rates in ungulate feces sampled in winter. Mol. Ecol. Notes 4: 772-775

MCKELVEY, K.S. – PEARSON, D.E. (2001): Population estimation with sparse data: the role of estimators versus indices revisited. Can. J. Zool 79, 1754-1765

MENKENS, G.E. – ANDERSON, S.H. (1988): Estimation of small-mammal population size. Ecology 69, 1952-1959

MILLER, C.G. – JOYCE, P. – WAITS, L.P. (2005): A new method for estimating the size of small populations from genetic mark-recapture data. Mol. Ecol. 14: 1991 - 2005

MÜLLER, T. – TEUFFERT, J. – ZIEDLER, K. – POSSARDT, C. – KRAMER, M. – STAUBACH, C. – CONRATHS, F.J. (1998): Pseudorabies in the European wild boar from Eastern Germany.- Journal of Wildlife Diseases 34, 251-258

MURPHY, M. – KENDALL, K. – ROBINSON, A. – WAITS, L.P. (2007): The impact of time and field conditions on brown bear (*Ursus arctos*) faecal DNA amplification. Conserv. Gen. 8: 1219-1224

OTIS, D.L. – BURNHAM, K.P. – WHITE, G.C. – ANDERSON, D.R. (1978): Statistical inference forv capture-recapture experiments. Wildl. Monogr. 107, 97 pp.

PALOMARES, F. – GODOY, J.A. – PIRIZ, A. – O'BRIEN, S.J. – JOHNSON, W.E. (2002): Faecal genetic analysis to determine the presence and distribution of elusive carbivores: design and feasibility for the Iberian lynx. Mol. Ecol. 11, 2171-2182

PIGGOTT, M.P. (2004): Effect of sample age and season of collection on the reliability of microsatellite genotyping of faecal DNA. Wildlife Research 31, 485-493

PIGGOTT, M.P. – TAYLOR, A.C. (2003): Remote collection of animal DNA and its applications in conservation management and understanding the population biology of rare and cryptic species. Wildl. Res. 30: 1-13

PUECHMAILLE, S.J. – PETIT, E.J. (2007): Empirical evaluation of non-invasive capture-mark-recapture estimation of population size based on a single sampling session.- J. Appl. Ecol. 44: 843 – 852

REGNAUT, S. – LUCAS, F.S. – FUMAGALLI, L. (2006): DNA degradation in avian faecal samples and feasibility of non-invasive genetic studies of threatened capercaillie populations. Conserv. Genet. 7, 449-453

REIS, M. (2006): Schalenwildbewirtschaftung im Forstamt Hinterweidenthal (Pfälzerwald) in den Jagdjahren 1999/00 bis 2005/06. Diploma Thesis, University for Applied Forest Sciences Rottenburg, Germany

SANTINI, A. – LUCCHINI, V. – FABBRI, E. – RANDI, E. (2007): Ageing and environmental factors affect PCR success in wolf (*Canis lupus*) excremental DNA samples. Mol. Ecol. Notes 7, 955 - 961

SCHEPPERS, T.L.J. – FRANTZ, A.C. – SCHAUL, M. – ENGEL, E. – BREYNE, P. – SCHLEY, L. – ROPER, T.J. (2007): Estimating social group size of Eurasian badgers *Meles meles* by genotyping remotely plucked single hairs. Wildl. Biol. 13: 195-207

SMITH, D.A. – RALLS, K. – CYPHER, B. – MALDONADO, J.E. (2005): Assessment of scat-detection dog surveys to determine kit fox distribution. Wildl. Soc. Bull.33, 897-904

SOLBERG, K.H. – BELLEMAIN, E. – DRAGESET, O.-M. – TABERLET, P. – SWENSON, J.E. (2006): An evaluation of field and non-invasive genetic methods to estimate brown bear (*Ursus arctos*) population size. Biol. Conserv. 128: 158–168

TOTTEWITZ, F. – STUBBE, – AHRENS, M. – DOBIÀS, K. – GORETZKI, J. – PAUSTIAN, K.-H. (1998): Die Losungszählung als Methode der Bestandesschätzung von wiederkäuenden Schalenwildarten. Z. Jagdwiss. 42, 111-122 (in german with english summary)

WHITE, G.C. – BURNHAM, K.P. – OTIS, D.L. – ANDERSON, D.R. (1978): User's Manual for Program CAPTURE, Utah State Univ. Press, Logan, Utah.

WHITE, G.C. – ANDERSON, D.R. – BURNHAM, K.P. – OTIS, D.L. (1982): Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory, New Mexico, LA-8787-NERP, 235 pp.

WOODS, J.G. – PAETKAU, D. – LEWIS, D. – MCLELLAN, B.N. – PROCTOR, M. – STROBECK, C. (1999): Genetic tagging of free-ranging black and brown bears. Wildl. Soc. Bull. 27: 616-627