Non-invasive genetic approaches to estimate ungulate population sizes in the Palatinate Forest, south-west Germany

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by

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Thesis abstract

Many populations of large ungulates are of management or conservation concern. In the Palatinate Forest in south-west Germany, wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) both occur in presumably large, but *de facto* unknown densities. For a sustainable management of both species, reliable and accurate population estimates are needed. Non-invasive genetic methods represent a powerful tool for wildlife management because animals can be monitored without physical capture or other human inference. They are mostly based on samples of hair, feathers or faeces which are genotyped in order to allow discrimination between individuals. One application of non-invasive genetic methods is estimation of population size.

In the study at hand, a non-invasive genetic approach for the estimation of wild boar and red deer population size is developed, tested in the field and evaluated. The first tests on wild boar relied on hair sampling using baited hair traps, which turned out to be not suitable for this species due to behavioural differences depending on age and group status. Thereafter, for wild boar as well as for red deer, faeces sampling along transect lines was tested and applied, and the collected samples were used for population estimation after genotyping.

For both species, population densities were derived from the estimated population sizes by augmenting the transect grid area by a buffer because the study area can not be considered as geographically closed. For wild boar, the buffer width was determined using own radio- and GPS telemetry data collected in the study area. In case of red deer, telemetry data from the neighbouring French Vosges were used. The estimated wild boar population densities were 4.1 (2.8 – 5.9) to 4.4 (3.0 – 6.4) wild boar per km² (dependent on the estimation approach is used) and for 2007 9.1 (5.6 – 11.4) to 9.6 (5.8 – 12.3) wild boar per km². For red deer, population sizes and thus densities were calculated separately for both sexes. The estimated red deer density for 2010 was 1.24 (0.98 – 1.95) males and 1.92 (1.35 – 3.84) females per km².

For both species, the estimated population densities were considerably larger than expected, taking into account the hunting bag statistics and – for red deer – spotlight counts. Harvest as the main management measure seems not sufficient for both species to regulate population sizes, thus the management plans for the study area should be revised.

The method presented in this study appears as a promising alternative to traditional methods like hunting bag statistics of pellet counts because it yields absolute population sizes, and thus allows a quantitative evaluation of the success of management measures.

In future research, the topic of population closure should be addressed in detail. Furthermore, the sample size should be increased for both species – in particular for wild boar – in order to further increase accuracy and precision of the population estimates.

Thesis review

Introduction

The assessment of population size and its changes over time is essential for the management of wildlife populations (Smart et al. 2004). This holds particularly true for densely populated and intensively agriculturally used areas, which is the case in most parts of Germany as well as in other countries in Central Europe. In such areas, wildlife habitat is often fragmented and conflicts between the needs of humans and wildlife are frequent. Knowledge about the abundance of animals allows an evaluation of the efficiency of management measures, on the one hand for animal conservation in case of rare or endangered species and on the other hand for population control in case of common or overabundant species.

Traditional methods for the estimation of animal abundance which have been applied to ungulate populations are e.g. analysis of hunting bags (Boitani et al. 1995a, Acevedo at al. 2007), direct sightings (Groot Bruinderink & Hazebroek 1995, Mysterud et al. 2007) and counts of faeces (Bailey & Putman 1987, Vicente et al. 2004). These methods merely yield indices of population size or dynamics, which allow less fine-grained conclusions on management questions compared to absolute numbers (Boitani et al. 1995, Monaco et al. 2004). The capture-mark-recapture (CMR) approach can result in absolute population estimates (Otis et al. 1978, Seber 1982, Pollock et al. 1990). However, capturing and marking large ungulates is arduous and costly. Furthermore, capture and recapture probabilities may vary greatly between individuals, being influenced by age, sex, social status, and individual experiences (Baber & Coblentz 1986). This can lead to severely biased population estimates (White et al. 1982). Behavioural responses to capture (‘trap happiness’ or ‘trap shyness’) can result in additional bias. Thus, traditional CMR has rarely been applied for the monitoring of large ungulate populations.

In this context, non-invasive genetic methods represent a powerful tool because animals can be monitored without physical capture or other human interference (Beja-Pereira et al. 2009). Non-invasive genetic methods are mostly based on hair,

feathers or faeces as sources of DNA. Hair, feather or faeces samples can be genotyped, allowing discrimination between different individuals. An individual genotype can be used as a natural 'mark', so that non-invasively obtained data can be analyzed within a CMR framework without physically capturing animals (Woods et al. 1999). In recent years, non-invasive genetic methods have been applied for population estimation in a range of mammal species, among them grizzly *Ursus arctos* and black bears *Ursus americanus* (Boulanger et al. 2004, Settlage et al. 2008), wombats *Lasiorhinus kreftii* (Banks et al. 2003), otters *Lutra lutra* (Prigioni et al. 2006), and African Elephants *Loxodonta africana* (Eggert et al. 2003).

In this thesis, a non-invasive genetic population estimation approach is established, applied and evaluated for two ungulate species, the wild boar (*Sus scrofa*) and the red deer (*Cervus elaphus*) in a study area situated in the Palatinate Forest, south-west Germany. Both species are of management concern in the study area (see below) and thus for both there is a need for reliable population size estimates.
Issues that were addressed particularly are:

- Is the sample size which can be obtained with a feasible effort sufficient for accurate estimation of wild boar and red deer population sizes?
- Do the genotyping and error-checking protocols allow reliable discrimination between different individuals?
- Are there individual or sex-based heterogeneities, closure violation or other sources of bias detectable which can compromise population estimation?
- What can be deduced from the resulting estimated population sizes concerning the wild boar and red deer management in the study area?

Studied species

WILD BOAR

In Central Europe, the wild boar is one of the species which has come into focus of wildlife management and also of the public perception. One main reason for this is that wild boar populations have increased rapidly during the last three to four decades in many parts of Europe (Acevedo et al. 2007, Bieber & Ruf 2005, Saez-Royuela & Telleria 1986). Among the consequences of this population increase are rises in agricultural and other damages (Schley et al. 2008). Furthermore, wild boar play a role

in the spread of infectious diseases like the classical swine fever, viral agents such as Aujeszky's disease and of bacteria like the *Mycobacterium tuberculosis* complex (Kaden 1998, Acevedo et al. 2007). This results in an increased need for a sustainable management of wild boar populations (Sweitzer et al. 2000). The main management measure for wild boar in most regions is harvest. However, until now it has not been possible to control the efficiency of harvest in regulating wild boar populations on a quantitative basis because absolute population estimates were lacking for most regions. Furthermore, reliable and accurate population estimates are important for epidemiological reasons because the spread of the classical swine fever and other diseases is related to wild boar density (Artois et al. 2002).

RED DEER

For red deer like for other large ungulates, two extremes (as well as the whole spectrum in between) exist: Some subspecies or populations are endangered and of conservation concern (e.g. the Barbary red deer *Cervus elaphus barbarus*), whereas others are overabundant (Barrio 2007, Haji et al. 2008). High densities of red deer can cause considerable damage through e.g. bark stripping or browsing (Allombert et al. 2005, Putman & Moore 1998). Red deer densities have increased in many parts of Europe (Gordon et al. 2004; Milner et al. 2006; Mysterud et al. 2007). From a silvicultural point of view, an effective management of deer populations is necessary in such cases (Ward 2005). However, estimating population sizes and dynamics for red deer is a difficult task. Deer populations in forested areas are particularly difficult to survey because direct counts are not feasible and indirect methods like e.g. pellet counts yield imprecise results (as reviewed in Smart et al. 2004). Thus for many regions in Europe reliable census data for red deer are not available, even though they are crucial in order to establish efficient and sustainable management plans (Milner et al. 2006).

In most federal states of Germany, red deer populations are restricted to assigned, mostly forested regions, habitat patches thus being highly fragmented. About 23% of the countries total area is designated deer area (Kinser et al. 2010). Populations are in general harvested, the evaluation of management measures and validation of the harvest quotas are until now mostly based on hunting bag statistics and/ or browsing

surveys. In this context, absolute population estimates can represent a quantitative and reliable data base.

Genetic methods

Non-invasive genetic methods have been applied for several different purposes, among them studies of population size and individual movement, wildlife forensic cases as well as studies of population genetic structure and gene flow (Beja-Pereira et al. 2009). Until now, in most cases the basis for identification of individual animals are gene segments called microsatellites or short tandem repeats (STR). Microsatellites are repeating sequences of one to six base pairs of genomic DNA (Ellegren 2004). The number of repeats varies between individuals and thus a sufficient number of analysed microsatellites (dependent on their degree of polymorphism) can allow the discrimination between individuals. The amount of target DNA in hair and faeces is often very low. The use of these non-invasive sources of DNA has been made possible through the discovery of the Polymerase Chain Reaction (PCR) which allows amplification of minute amounts of DNA using a thermostable polymerase (Taberlet & Luikart 1999). The analysis of non-invasive samples nevertheless implies several pitfalls due to low DNA quantity and quality. DNA amplification can be hindered by PCR inhibitors particularly in faeces, and genotyping errors like allelic dropout and false alleles can occur (Taberlet & Luikart 1999, McKelvey & Schwartz 2004). Genotyping errors can lead to false identification of individuals and therefore bias population estimates. Most types of genotyping errors result in an overestimation of population size. Consequently, careful error-checking protocols should be applied when non-invasive samples are analysed, and often several repeats of the analysis are necessary to yield a reliable genotype (Paetkau 2003).

Study area

The area in which the complete study was carried out is approximately 100 km² of size and situated in the Palatinate Forest, in the federal state of Rhineland-Palatinate in south-west Germany. The area has been designated for wildlife research in 2005. The area is almost entirely state-run concerning hunting and forestry. It is part of the Biosphere Reserve Rhineland-Palatinate – Northern Vosges and contains a large Biosphere core area. 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), beech forest (Luzulo-Fagetum) being the predominant native plant community. Several small settlements with surrounding open areas lie in 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: roe deer *Capreolus capreolus,* red deer, and wild boar. The annual harvest of wild boar between 1999 and 2009 averages 2.4 individuals per km² (Range: 1.14 to 5.23 individuals per km² and year, whereas the average red deer hunting bag from 1999 to 2009 is 1.0 per km² and year (minimum 0.7, maximum 1.3)).

Data collection

For the study at hand, data collection started in January 2006 with the capture, marking and observation of wild boar via radio- and GPS-telemetry. The aim of telemetric observation was to assess space and habitat use as well as movement distances of the animals to develop and validate a non-invasive hair and faeces sampling design. Between January 2006 and January 2008, we observed 19 wild boar for periods between one and 20 month.

For non-invasive sampling, we first tested a hair sampling design based on baited hair traps made of barbed wire (cf. Chapter 2). Hair sampling was carried out between April and August 2006. The first faeces sampling trial for wild boar was conducted in December 2006, followed by five more trials until March 2010 (cf. Chapter 3 and 4). Red deer faeces sampling was carried out in March 2010 (cf. Chapter 5). First tests of red deer faeces sampling had been carried out before in March 2009. Attempts were made to capture red deer and equip them with GPS transmitters in order to observe their space use, but they were not successful. Thus, for the determination of the red deer transect design, telemetry data from red deer in the neighbouring French Vosges were used.

Wild boar samples collected in December 2006 and December 2007 were genotyped by K. Kolodziej (University of Koblenz-Landau, Germany) using four microsatellite loci and a Y-linked marker for sex determination. For red deer, the 2010 samples

were analysed using seven microsatellite loci plus a sex marker. Analyses were carried out by B. Spielberger (SEQ-IT GmbH und Co. KG, Kaiserslautern, Germany).

Thesis structure

Chapter 1: Individual heterogeneity as a pitfall in population estimates based on non-invasive genetic sampling – review and recommendations

In this chapter, the theoretical background for non-invasive genetic population estimation is addressed, with a focus on the issue of heterogeneous detection probabilities. The relevant literature is reviewed to obtain an overview over non-invasive genetic population estimation studies and the handling of problems due to individual heterogeneity.

Chapter 2: Can hair traps sample wild boar (*Sus scrofa*) randomly for the purpose of non-invasive population estimation?

The non-invasive collection of wild boar hair samples using baited hair traps is tested. The behaviour of wild boar at the hair traps is observed with video cameras to assess whether a random sampling with respect to sex, age class and group status is feasible.

Chapter 3: 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

Wild boar faeces are collected along transect lines in the Palatinate Forest and genotyped. The obtained sample size is evaluated with respect to CMR requirements. The cost effectiveness and the outcome of the study are compared to other non-invasive population studies.

Chapter 4: Estimating wild boar (*Sus scrofa* L.) population size using faecal DNA and capture-recapture modelling

Population estimates based on genotypes from wild boar faeces are presented for two consecutive years (2006 and 2007). The comparison between the years indicates a strong increase in population size. The resulting population densities and estimated reproductive output are compared to the hunting bag in the study area in order to evaluate the effectiveness of the local wild boar management.

Chapter 5: Estimating red deer (*Cervus elaphus*) population size based on non-invasive genetic sampling

A non-invasive genetic population estimation approach is developed and applied for red deer. The sampling scheme and genotyping protocol are adapted to this species. Because of the sexual dimorphism of red deer, population estimates are calculated separately for the two sexes. The assumption of population closure is tested. The resulting population densities are set into context with the local red deer management.

Results

WILD BOAR

For wild boar, the non-invasive hair sampling using baited hair traps seemed not suitable for the purpose of population estimation. The analysis of 216 videotaped wild boar visits to hair traps revealed distinct behavioural differences dependent on age class and group status of the wild boar (cf. Chapter 2). This heterogeneity in behaviour would most probably result in a heterogeneous sampling behaviour and thus in biased population estimates.

Therefore, we pursued the sampling of faeces instead of hair. The collection of faeces is – in contrast to the use of baited hair traps – a 'passive' sampling strategy which means that the animals do not have to actively approach a sampling station.
Altogether, we collected more than 1500 wild boar faeces over four years. Of the samples collected in December 2006 (141 samples) and those collected in December 2007 (326 samples), DNA was extracted and 89 and 155 respectively were genotyped successfully (cf. Chapter 3 and 4). Both data sets are sparse with respect to the detection probability of the wild boar, which exacerbates the selection of the most appropriate population estimation model. I chose the most conservative approach for inferences concerning the wild boar management because non-invasive genetic population estimates are often in danger of being biased high due to heterogeneity and genotyping errors. Population estimates were calculated from the two data sets using program MARK (White & Burnham 2001). The population densities were then calculated by augmenting the transect grid area with a buffer. The density for 2006 is 4.1 (2.8 – 5.9) to 4.4 (3.0 – 6.4) wild boar per km² (dependent

on which estimation approach is used, cf. Chapters 3 and 4) and for 2007 9.1 (5.6 – 11.4) to 9.6 (5.8 – 12.3) wild boar per km² (cf. Chapter 4).

I used the lower confidence intervals of the estimated population densities to calculate an estimate of the reproductive output in the studied population (with a reproductive rate of 200% based on literature data, Gethöffer et al. 2007). A comparison of the hunting bag realised in the study area to the estimated population density and reproductive output indicates for both years that the present hunting regime does not achieve a reduction of the studied wild boar population (which had been the aim of the forestry in charge). In fact, the hunting bag corresponded to merely approximately 35% of the estimated reproductive output and thus would not be able to stop a population increase.

RED DEER

We collected 1128 red deer faeces in March 2010. From these samples, DNA was extracted, the content of target DNA was determined via real-time quantitative PCR, and 398 were genotyped successfully. The 398 genotypes represented 247 different red deer individuals. The model averaged population estimate based on program MARK was calculated for males and females separately and yielded an estimated population of 161 (126 – 252) male and 249 (174 – 495) female red deer in the study area. To test for violation of the closure assumption, I used Pradel models for open populations (Boulanger & MecLellan 2001). The results of this test indicated that there had been some closure violation in our study area. Thus, I augmented the transect grid area by a 750 m buffer, corresponding to a seasonal male red deer home range radius for calculation of population densities. The resulting densities based on an area of 129 km² were 1.24 (0.98 – 1.95) male and 1.92 (1.35 – 3.84) female red deer per km². The issue of population closure will have to be addressed in more detail in future research.

The red deer numbers estimated in this study exceed considerably those previously assumed based on hunting bag statistics or spotlight counts. Therefore, the red deer management plan for the area should be reconsidered.

Parts of this thesis have been published or submitted for publication to scientific journals:

Ebert, C., Knauer, F., Storch, I., Hohmann, U. (2010): Individual heterogeneity as a pitfall in population estimates based on non-invasive genetic sampling: a review and recommendations. Wildlife Biology 16, 225-240. [Chapter 1]
C. Ebert conducted the literature research, analysed the data and wrote the manuscript. F. Knauer provided part of the statistic analysis and revised the manuscript. I. Storch suggested the topic and revised the manuscript. U.Hohmann revised the manuscript.

Ebert, C., Huckschlag, D., Schulz, H.K., Hohmann, U. (2010): Can hair traps sample wild boar (*Sus scrofa*) randomly for the purpose of non-invasive population estimation? European Journal of Wildlife Research 56, 583-590. [Chapter 2]
C. Ebert conducted the hair sampling trials, analysed the data and wrote the manuscript. D. Huckschlag provided help with video camera setup and study design. H.K. Schulz helped with the study design. U. Hohmann provided ideas for study design and topic and revised the manuscript.

Ebert, C., Kolodziej, K., Schikora, T., Schulz, H.K., Hohmann, U. (2009): Is non-invasive genetic population estimation via faeces sampling feasible for abundant mammals with low defecation rates? A pilot study on freeranging wild boar (*Sus scrofa*) in South-West Germany. Acta Silvatica et Lignaria Hungarica 5, 167-177 [Chapter 3]
C. Ebert conducted the faeces sampling trial, analysed the data, did the population estimation modelling and wrote the manuscript. K. Kolodziej genotyped the faeces samples. T. Schikora developed the transect design and collected faeces samples. H.K. Schulz and U. Hohmann provided help with the study design and revised the manuscript.

Ebert, C., Kolodziej, K., Schulz, H.K., Hohmann, U.: Estimating wild boar (*Sus scrofa* L.) population size using faecal DNA and capture-recapture modelling. Submitted to Wildlife Biology. [Chapter 4]
C. Ebert conducted the faeces sampling trials, analysed the data, did the population estimation modelling and wrote the manuscript. K. Kolodziej did the genetic analyses. H.K. Schulz helped with the study design and the genotyping protocol. U. Hohmann helped with the study design and revised the manuscript.

Ebert, C., Marell, R., Rahlfs, M., Spielberger, B., Hohmann, U.: Estimating red deer (*Cervus elaphus*) population size based on non-invasive genetic sampling. Submitted to the European Journal of Wildlife Research. [Chapter 5]

C. Ebert conducted the faeces sampling trials, analysed the data, did the population estimation modelling and wrote the manuscript. R. Marell helped with the genotyping protocol and the genetic methods and revised the manuscript. M. Rahlfs helped with the faeces sampling and study design and revised the manuscript. B. Spielberger did the genetic analysis of the faeces samples. U.Hohmann provided support for the development of the study design and the collection of samples and revised the manuscript.

Zusammenfassung

Viele Populationen grosser Huftiere sind entweder management- oder artenschutzrelevant. Im in Südwest-Deutschland gelegenen Pfälzerwald kommen sowohl Wildschweine (*Sus scrofa*) als auch Rothirsche (*Cervus elaphus*) in mutmaßlich großen, aber letztendlich unbekannten Dichten vor. Für ein nachhaltiges Management beider Arten sind verlässliche und akkurate Populationsschätzungen nötig. Nicht-invasive genetik-basierte Methoden repräsentieren in diesem Zusammenhang ein nützliches Instrument für das Wildtiermanagement, da sie es erlauben, Tiere zu erfassen, ohne sie zu fangen oder anderweitig zu beeinflussen. Nicht-invasive Methoden arbeiten meist mit Haar-, Feder- oder Kotproben, die genotypisiert werden und so eine Unterscheidung zwischen Individuen ermöglichen. Eine der Anwendungen nicht-invasiver Methoden ist die Populationsschätzung.

In der vorliegenden Studie wurde ein nicht-invasiver genetik-basierter Ansatz für die Populationsschätzung bei Wildschweinen und Rothirschen entwickelt, im Freiland getestet und anschließend evaluiert. In ersten Versuchen am Wildschwein wurde die Haarbeprobung mittels beköderter „Haarfänger“ getestet. Dabei zeigte sich, dass die Methode für diese Tierart für den Zweck der Populationsschätzung nicht geeignet ist. Grund hierfür waren signifikante alters- und gruppenstatusabhängige Verhaltensunterschiede bei der Beprobung. Im Folgenden wurde sowohl für Wildschweine als auch für Rothirsche die Kotbeprobung entlang von Transektlinien getestet und angewandt; die so gewonnenen Proben wurden genotypisiert und zum Berechnen von Populationsschätzungen verwendet.

Für beide Tierarten wurden aus den geschätzten Populationsgrößen resultierend Populationsdichten berechnet. Dazu wurde die von den Transekten abgedeckte Fläche jeweils um einen Puffer vergrößert, da das Untersuchungsgebiet nicht als geografisch geschlossen bezeichnet werden kann. Die Breite des Puffers wurde für Wildschweine anhand von Radio- und GPS- Telemetriedaten aus dem Untersuchungsgebiet ermittelt. Im Fall des Rothirsches wurden dazu Telemetriedaten aus den benachbarten französischen Vogesen verwendet. Die geschätzten Wildschwein-Populationsdichten betrugen für das Jahr 2006 4,1 (2,8 – 5,9) bis 4,4

(3,0 – 6,4) Tiere pro km² (in Abhängigkeit vom verwendeten Schätzmodell) und für das Jahr 2007 9,1 (5,6 – 11,4) bis 9,6 (5,8 – 12,3) Tiere pro km². Für Rothirsche wurden die Populationsgrößen und damit auch die -dichten für beide Geschlechter getrennt ermittelt. Die geschätzte Rothirschdichte für das Jahr 2010 betrug 1,24 (0,98 – 1,95) männliche und 1,92 (1,35 – 3,84) weibliche Tiere pro km².

Für beide Tierarten sind die geschätzten Populationsdichten erheblich höher als zuvor angenommen, da bislang lediglich die Jagdstreckenstatistik und – im Fall des Rothirsches – Scheinwerferzählungen als Anhaltspunkt genommen werden konnten. Die Jagd als hauptsächlich angewandte Managementmassnahme erscheint aufgrund der vorliegenden Schätzungen für beide Tierarten nicht ausreichend, um eine Regulierung der Populationen zu gewährleisten. Daher sollten die Managementpläne für das Untersuchungsgebiet neu überdacht werden.

Die hier vorgestellte Methode stellt eine viel versprechende Alternative zu den traditionell angewandten Methoden wie z.B. Jagstreckenstatistiken oder Losungszählverfahren dar, da sie absolute Populationszahlen ergibt und damit eine quantitative Bewertung des Erfolgs von Managementmassnahmen ermöglicht. Die Methode könnte auch auf andere Huftierarten übertragen angewandt werden.

In zukünftiger Forschung sollte die Frage der Geschlossenheit der Population im Detail erörtert werden. Weiterhin sollte die Stichprobengröße für beide Tierarten – insbesondere für Wildschweine – vergrößert werden, um die Genauigkeit und Präzision der Populationsschätzungen weiter zu erhöhen.

Übersicht

Einleitung

Das Bestimmen der Populationsgröße sowie deren Veränderung über die Zeit ist essenziell für das Management von Wildtierpopulationen (Smart et al. 2004). Dies gilt insbesondere für dicht besiedelte und intensiv landwirtschaftlich genutzte Gebiete, was auf große Teile Deutschlands und anderer mitteleuropäischer Länder zutrifft. In solchen Gebieten sind Wildtierlebensräume oftmals fragmentiert, und Konflikte zwischen den Bedürfnissen von Mensch und Wildtieren sind häufig. Kenntnis über die Abundanz von Tieren ermöglicht eine Bewertung der Effizienz von Management-Maßnahmen – einerseits für den Artenschutz im Falle von seltenen oder bedrohten Tierarten, andererseits zur Populationskontrolle bei verbreiteten oder sehr häufigen Arten.

Traditionell bei Huftieren angewandte Methoden zur Bestandesschätzung sind z.B. Jagdstreckenanalysen (Boitani et al. 1995a, Acevedo at al. 2007), direkte Zählungen (Groot Bruinderink & Hazebroek 1995, Mysterud et al. 2007) sowie Losungszählungen (Bailey & Putman 1987, Vicente et al. 2004). Diese Methoden erzielen lediglich Indices der Populationsdichte oder Populationsentwicklung, was, verglichen mit absoluten Schätzungen dr Populationsgröße, weniger detaillierte Aussagen in Bezug auf Management-Fragestellungen erlaubt (Boitani et al. 1995, Monaco et al. 2004). Der Capture-Mark-Recapture- (CMR) Ansatz ermöglicht absolute Populationsschätzungen (Otis et al. 1978, Seber 1982, Pollock et al. 1990). Das Fangen und Markieren großer Ungulaten ist allerdings schwierig und aufwändig. Zudem können die Fang- und Wiederfangwahrscheinlichkeit stark variieren, u.a. nach Alter, Geschlecht, sozialem Status und individueller Vorerfahrungen (Baber & Coblentz 1986). Dies kann starke Fehler in den Populationsschätzungen bewirken (White et al. 1982). Verhaltensreaktionen auf den Fang (sog. „trap happiness“ oder „trap shyness“) können Schätzungen zusätzlich beeinflussen. Der traditionelle CMR-Ansatz ist daher nur selten für Populationen großer Huftiere angewandt worden.

In diesem Zusammenhang stellen nicht-invasive genetische Methoden eine wichtige Alternative dar, da Tiere hierbei ohne physischen Fang oder andere Beeinträchtigungen durch den Menschen erfasst werden können (Beja-Pereira et al. 2009). Nicht-invasive genetische Methoden basieren meist auf Haaren, Federn oder Kot als DNA-Quelle. Durch das Genotypisieren von Haar-, Feder- oder Kotproben kann zwischen einzelnen Individuen unterschieden werden. Ein individueller Genotyp kann somit als natürliche Markierung dienen, so dass nicht-invasiv gewonnene Daten in einem CMR-System ausgewertet werden können, ohne dass dafür tatsächlich Tiere gefangen wurden (Woods et al. 1999). In den letzten Jahren sind nicht-invasive genetische Methoden zur Populationsschätzung bei verschiedenen Säugetierarten angewandt worden, darunter Grizzly- und Schwarzbären (*Ursus arctos* und *Ursus americanus*; Boulanger et al. 2004, Settlage et al. 2008), Wombats (*Lasiorhinus kreftii*; Banks et al. 2003), Fischotter (*Lutra lutra*; Prigioni et al. 2006) und Afrikanische Elefanten (*Loxodonta africana*; Eggert et al. 2003).

In der vorliegenden Arbeit wurde eine nicht-invasive genetische Methode zur Populationsschätzung von Wildschweinen (*Sus scrofa*) und Rothirschen (*Cervus elaphus*) in einem Untersuchungsgebiet im Pfälzerwald entwickelt, angewandt und evaluiert. Beide Tierarten sind im Untersuchungsgebiet management-relevant (siehe Abschnitt „Untersuchte Tierarten“), und belastbare Populationsschätzungen werden dringend benötigt.

In der Arbeit wurden insbesondere folgende Fragen behandelt:

- Ist die Stichprobengröße, die mit einem vertretbaren Aufwand gewonnen werden kann, ausreichend für eine präzise Populationsschätzung von Wildschwein und Rotwild?
- Erlauben das Genotypisierungsprotokoll und die Genotypisierungsfehler-Tests eine verlässliche Unterscheidung zwischen Individuen?
- Gibt es individuelle oder geschlechtsbezogene Heterogenitäten in der Detektionswahrscheinlichkeit, ist die Geschlossenheit der Population gewährleistet und sind andere Fehlerquellen vorhanden, die eine Populationsschätzung beeinträchtigen können?
- Was kann man aus den geschätzten Populationsgrößen für das Wildschwein- und Rotwildmanagement im Untersuchungsgebiet folgern?

Untersuchte Tierarten

WILDSCHWEIN

In Mitteleuropa ist das Wildschwein sowohl hinsichtlich des Managements als auch in der öffentlichen Wahrnehmung in den letzten Jahren zunehmend in den Fokus gerückt. Ein Hauptgrund hierfür ist, dass Wildschweinpopulationen in weiten Teilen Europas in den letzten drei bis vier Jahrzehnten stark angestiegen sind (Acevedo et al. 2007, Bieber & Ruf 2005, Saez-Royuela & Telleria 1986). Die Folgen dieses Populationsanstiegs sind u.a. erhöhte landwirtschaftliche Schäden (Schley et al. 2008). Zudem spielen Wildschweine eine Rolle bei der Verbreitung von Infektionskrankheiten wie der Klassischen Schweinepest, von Viren wie z.B. dem Aujezsky-Virus und von Bakterien wie dem *Mycobacterium tuberculosis* – Komplex (Kaden 1998, Acevedo et al. 2007). Dadurch ist ein nachhaltiges Management für Wildschweinpopulationen zunehmend notwendig geworden (Sweitzer et al. 2000). Die hauptsächlich angewandte Management-Massnahme ist im Falle des Wildschweins in den meisten Regionen die Jagd. Es ist allerdings bislang nicht möglich gewesen, die Effektivität der Jagd bei der Regulierung von Wildschwein-Populationen auf einer quantitativen Basis zu überprüfen, da absolute Populationszahlen für die meisten Gebiete fehlen. Verlässliche und genaue Populationsschätzungen sind zudem auch aus epidemiologischen Gründen von Bedeutung, da die Ausbreitung der Klassischen Schweinepest und anderer Krankheiten mit der Wildschweindichte in Zusammenhang steht.

ROTHIRSCH

Für den Rothirsch, wie auch für andere große Huftierarten, existieren zwei Extreme (sowie die gesamte Bandbreite dazwischen): Manche Unterarten oder Populationen sind gefährdet und bedürfen des Schutzes (z.B. *Cervus elaphus barbarus*), andere wiederum sind sehr häufig (Barrio 2007, Haji et al. 2008). Hohe Rothirsch-Dichten können – vor allem in Waldgebieten – Schäden durch Rindenschäle und Verbiss bewirken (Allombert et al. 2005, Putman & Moore 1998). Die Rothirsch-Dichten sind in vielen Teilen Europas angestiegen (Gordon et al. 2004; Milner et al. 2006; Mysterud et al. 2007). Aus forstwirtschaftlicher Sicht ist in solchen Fällen ein effektives Rothirsch-Management nötig, um den Einfluss auf den Wald zu begrenzen (Ward 2005). Das Ermitteln von Populationsgröße und –dynamik ist für diese Tierart schwierig. Besonders Rothirschpopulationen in Waldgebieten sind schwer zu erfassen, da hier direkte Zählungen nicht durchführbar sind und indirekte Methoden

wie z.B. Losungszählungen ungenaue Ergebnisse liefern (siehe Überblick in Smart et al. 2004). Daher sind für viele Regionen Europas keine verlässlichen Zensus-Daten verfügbar, obwohl sie äußerst wichtig sind für das Erstellen nachhaltiger Management-Pläne (Milner et al. 2006).

In den meisten deutschen Bundesländern sind Rothirschgebiete gesetzlich beschränkt auf festgelegte, meist bewaldete Areale, weshalb Verbreitungsgebiete stark fragmentiert sind. Etwa 23% der Gesamtfläche Deutschlands sind Rothirsch-Gebiet (Kinser et al. 2010). Die dortigen Populationen werden in der Regel bejagt, eine Evaluation der Management-Maßnahmen und eine Validierung der Abschussquoten erfolgt bislang in der Regel auf Basis von Jagdstreckendaten und/ oder Verbissgutachten. Genaue Zensus-Daten in Form von absoluten Populations-schätzungen könnten hier eine quantitative und objektive Datengrundlage bilden.

Genetische Methodik

Nicht-invasive genetische Methoden wurden bislang für verschiedene Zwecke angewandt, darunter sind Populationsschätzung und Studien individuellen Bewegungsverhaltens, Wildtier-Forensik sowie Studien von genetischer Populationsstruktur und Genfluss (Beja-Pereira et al. 2009). Bis heute wurden in den meisten Fällen so genannte Mikrosatelliten (oder „short tandem repeats“, STR) als Grundlage für die Identifizierung einzelner Individuen verwendet. Mikrosatelliten sind sich wiederholende Sequenzen von ein bis sechs Basenpaaren genomischer DNA (Ellegren 2004). Die Anzahl Wiederholungen variiert zwischen Individuen, daher ermöglicht eine ausreichende Anzahl analysierter Mikrosatelliten (abhängig von ihrem Polymorphiegrad), zwischen Individuen zu unterscheiden.

Die Menge an verwertbarer Ziel-DNA ist in Haar- und Kotproben meist gering. Die Verwendbarkeit dieser nicht-invasiven DNA-Quellen wurde erst möglich durch die Erfindung der Polymerase-Kettenreaktion („polymerase chain reaction“, PCR), die eine Amplifizierung von sehr geringen Mengen an DNA durch die Verwendung einer thermostabilen Polymerase ermöglicht (Taberlet & Luikart 1999). Die Analyse nicht-invasiver Proben beinhaltet allerdings mehrere potentielle Problem- bzw. Fehlerquellen bedingt durch die geringe DNA- Menge und DNA- Qualität. Die DNA-Amplifizierung kann beispielsweise durch PCR- Inhibitoren behindert werden, und

Genotypisierungsfehler wie z.B. Allel-Ausfälle („allelic dropout“) und Falsch-Allele („false alleles“) können auftreten (Taberlet & Luikart 1999, McKelvey & Schwartz 2004). Genotypisierungsfehler können zu einer Fehlidentifikation von Individuen führen und dadurch Populationsschätzungen verfälschen. Die meisten Arten von Genotypisierungsfehlern führen zu einer Populationsüberschätzung. Daher sollten Genotypen besonders gründlich auf Fehler hin untersucht werden, wenn nicht-invasive Proben analysiert werden. Häufig sind mehrere Wiederholungen der PCR nötig, um einen verlässlichen Genotyp zu erhalten (Paetkau 2003).

Untersuchungsgebiet

Die gesamte Studie wurde in einem etwa 100 km² großen Gebiet im zentralen Pfälzerwald im Bundesland Rheinland-Pfalz durchgeführt. Das Gebiet ist seit 2005 als Wildforschungsgebiet ausgewiesen. Es liegt nahezu vollständig im Staatsforst, ist Teil des Biosphärenreservats Pfälzerwald-Nordvogesen und beinhaltet ein großes Biosphärenreservats-Kerngebiet. Es ist zu etwa 90% bewaldet (44% *Fagus sylvatica*, 26% *Pinus* sp., 10% *Picea abies*, 12% *Quercus petraea* and *Quercus robur*; Reis 2006), wobei Buchenwald (*Luzulo-Fagetum*) die vorherrschende Pflanzengesellschaft bildet. Mehrere kleinere Dörfer und Siedlungen mit umliegenden Freiflächen liegen im Umfeld des Wildforschungsgebiets. Die jährliche Durchschnittstemperatur ist 8-9 °C (Weiß 1993), die jährliche Niederschlagssumme beträgt im Mittel 600-1000 mm.

Drei Huftierarten kommen im Gebiet vor: Rehe (*Capreolus capreolus*), Rotwild und Wildschweine. Die jährliche Wildschwein-Jagdstrecke betrug zwischen 1999 und 2009 2,4 Tiere pro km² (zwischen 1,14 und 5,23 Tiere pro km² und Jahr). Die Rotwild-Jagdstrecke über den gleichen Zeitraum betrug im Durchschnitt 1,0 Tiere pro pro km² und Jahr (Minimum 0,7, Maximum 1,3).

Datenerhebung

Die Datenerhebung für die vorliegende Studie begann im Januar 2006 mit dem Fang, der Markierung, Besenderung und Beobachtung von Wildschweinen mittels VHF- und GPS- Sendern. Die telemetrische Überwachung der Tiere sollte dazu dienen, über ihre Raumbewegungen und Bewegungsdistanzen ein nicht-invasives Beprobungsdesign für Haare und Kot zu entwickeln und zu validieren. Zwischen

Januar 2006 und Januar 2008 wurden insgesamt 19 Wildschweine für Zeiträume zwischen einem und zwanzig Monaten telemetrisch überwacht.

Als nicht-invasive DNA-Quelle testeten wir zunächst die Haarbeprobung mittels beköderter „Haarfänger“, die aus Stacheldraht gefertigt wurden (siehe Kapitel 2). Die Haarbeprobungstests wurden zwischen April und August 2006 durchgeführt. Der erste Wildschwein-Kotbeprobungsversuch erfolgte im Dezember 2006, gefolgt von fünf weiteren Versuchen bis März 2010 (siehe Kapitel 3 und 4). Die Rotwild-Kotbeprobung wurde im März 2010 durchgeführt (siehe Kapitel 5). Rotwild-Losung wurde bereits 2009 versuchsweise gesammelt. Das Transekt-Design für die Rotwild-Beprobung wurde mangels eigener Telemetriedaten basierend auf Daten aus einem Rotwild-Telemetrieprojekt in den benachbarten Vogesen erstellt.

Die im Dezember 2006 und Dezember 2007 gesammelten Wildschwein-Kotproben wurden von K. Kolodziej (Universität Koblenz-Landau) unter Verwendung von vier Mikrosatelliten und einem Y-gebundenen Geschlechtsmarker genotypisiert. Die Rotwild-Proben aus dem Jahr 2010 wurden anhand von sieben Mikrosatelliten und einem Geschlechtsmarker genotypisiert. Die Analysen wurden von der Seq-it GmbH und Co. KG (Kaiserslautern) durchgeführt.

Aufbau der Arbeit

Kapitel 1: Individuelle Heterogenität als Fallstrick für Populationsschätzungen, die auf nicht-invasiver genetischer Beprobung basieren – ein Überblick und Empfehlungen

Dieses Kapitel befasst sich mit dem theoretischen Hintergrund für nicht-invasive genetik-basierte Populationsschätzung, der Schwerpunkt liegt dabei auf dem Problem der heterogenen Beprobungswahrscheinlichkeiten. Es wird ein Überblick über die themenbezogene Fachliteratur im Hinblick auf nicht-invasive Populationsstudien und den Umgang mit der Heterogenitätsproblematik gegeben.

Kapitel 2: Kann man mit „Haarfängern“ eine repräsentative Beprobung von Wildschweinen (*Sus scrofa*) zum Zwecke der nicht-invasiven Populationsschätzung erreichen?

Das nicht-invasive Gewinnen von Wildschwein-Haarproben mittels beköderter „Haarfänger“ wurde getestet. Das Verhalten der Tiere an den Beprobungsstationen

wurde mittels Videoüberwachung beobachtet und ausgewertet, um herauszufinden, ob eine repräsentative Erfassung im Hinblick auf die verschiedenen Geschlechter, Altersklassen und Gruppenzugehörigkeiten realisierbar ist.

Kapitel 3: Ist nicht-invasive genetik-basierte Populationsschätzung mittels Kotbeprobung praktikabel für häufig vorkommende Tierarten mit niedrigen Defäkationsraten? Eine Pilotstudie an frei lebenden Wildschweinen (*Sus scrofa*) im Südwesten Deutschlands

Wildschwein-Kot wurde im Pfälzerwald entlang von Transektlinien gesammelt und anschließend genotypisiert. Die erreichte Stichprobengröße wird im Hinblick auf die Erfordernisse für die Durchführung einer CMR- Populationsschätzung bewertet. Kosten und Effektivität der Studie werden mit anderen nicht-invasiven Populationsstudien verglichen.

Kapitel 4: Schätzen der Größe einer Wildschwein- Population (*Sus scrofa* L.) anhand von DNA auf Kotproben und mittels „Capture-Recapture"-Modellierung

In diesem Kapitel werden genetik-basierte Wildschwein-Populationsschätzungen aus zwei aufeinander folgenden Jahren (2006 und 2007) präsentiert. Der Vergleich zwischen den beiden Jahren deutet auf einen starken Populationsanstieg hin. Die daraus resultierenden Populationsdichten für das Untersuchungsgebiet sowie der daraus geschätzte reproduktive „Output" der Population werden mit der Jagdstrecke im Gebiet verglichen, um die Effektivität des dortigen Wildschwein-Managements bewerten zu können.

Kapitel 5: Populationsschätzung beim Rotwild (*Cervus elaphus*) aufgrund von nicht-invasiver genetischer Beprobung

Ein nicht-invasiver genetik-basierter Ansatz zur Populationsschätzung von Rotwild wird entwickelt und angewandt. Das Beprobungsdesign und Genotypisierungsprotokoll wurden an die Tierart angepasst. Wegen des ausgeprägten Sexualdimorphismus beim Rotwild werden Populationsschätzungen für beide Geschlechter getrennt berechnet. Die Annahme einer geschlossenen Population wird geprüft. Die berechneten Populationsdichten werden im Zusammenhang mit dem örtlichen Rotwildmanagement betrachtet.

Ergebnisse

WILDSCHWEIN

Die nicht-invasive Haarbeprobung mittels beköderter „Haarfänger“ erwies sich als ungeeignet für die Populationsschätzung bei Wildschweinen. Die Analyse von 216 per Videokamera aufgezeichneter Wildschwein-Besuche an „Haarfängern“ zeigte deutliche Verhaltens-Unterschiede abhängig vom Alter und von der Gruppenzugehörigkeit der Tiere (siehe Kapitel 2). Diese Heterogenität im Verhalten würde höchstwahrscheinlich auch heterogene Beprobungswahrscheinlichkeiten nach sich ziehen, was wiederum die Populationsschätzungen verfälschen würde. Infolgedessen haben wir anstatt der Haar- die Kotbeprobung als Methode weiterverfolgt. Das Sammeln von Kot ist – im Gegensatz zur Verwendung beköderter „Haarfänger“ – eine „passive“ Beprobungsstrategie, da die Tiere sich nicht aktiv einer Beprobungsstation nähern müssen.

Insgesamt wurden mehr als 1500 Wildschwein-Kotproben im Lauf der vier Jahre gesammelt. Von diesen wurden aus den Proben aus dem Dezember 2006 (141 Proben) und aus dem Dezember 2007 (326 Proben) die DNA extrahiert, und 89 bzw. 155 Proben konnten jeweils erfolgreich genotypisiert werden (siehe Kapitel 3 und 4). Beide Datensätze zeigen eine relativ geringe durchschnittliche Detektionswahrscheinlichkeit für die Wildschweine, was die Auswahl des am besten geeigneten Populationsschätzungsmodells erschwert. Ich habe mich im Hinblick auf die Verwendung der Schätzungen zum Beurteilen des Wildschwein-Managements eine sehr konservative Herangehensweise entschieden, da nicht-invasive genetik-basierte Populationsschätzungen aufgrund von Heterogenität und Genotypisierungsfehlern häufig eher zur Überschätzung der Populationsgröße neigen. Aus den beiden Datensätzen wurden mit dem Programm MARK (White & Burnham 2001) Populationsgrößen berechnet. Daraus resultierend habe ich Populationsdichten berechnet, die ich auf die durch einen Puffer erweiterte Untersuchungsfläche bezogen habe. Die für das Jahr 2006 ermittelte Populationsdichte beträgt zwischen 4,1 (2,8 – 5,9) und 4,4 (3,0 – 6,4) Wildschweine pro km² (in Abhängigkeit davon, welches Modell zur Populationsschätzung verwendet wurde, siehe Kapitel 3 und 4). Für 2007 beträgt die Dichte zwischen 9,1 (5,6 – 11,4) und 9,6 (5,8 – 12,3) Wildschweine pro km² (siehe Kapitel 4).

Ich habe die unteren Konfidenzintervalle der geschätzten Populationsdichten gewählt, um einen Schätzwert für den reproduktiven „Output“ der Population zu berechnen. Die dazu verwendete Reproduktionsrate (200%) basiert auf Literaturangeben (Gethöffer et al. 2007). Ein Vergleich zwischen der Jagstrecke im Untersuchungsgebiet mit der geschätzten Populationsdichte und der Reproduktion zeigt für beide Jahre, dass die im Gebiet ausgeübte Bejagung keine Reduktion der Wildschweinpopulation erreichen konnte (dies war vom zuständigen Forstamt beabsichtigt gewesen). In der Tat entspricht die Jagdstrecke nur etwa 35% der geschätzten Reproduktion, so dass auch der Zuwachs nicht abgeschöpft werden konnte und so auch ein weiteres Ansteigen der Population nicht hätte verhindert werden können. .

ROTWILD

Es wurden im März 2010 1128 Rotwild-Kotproben gesammelt. Aus diesen wurde DNA extrahiert, der Gehalt an Ziel-DNA wurde anhand einer quantitativen „real-time-“ PCR bestimmt, und 398 konnten erfolgreich genotypisiert werden. Die 398 Genotypen repräsentieren 247 verschiedene Rothirsch-Individuen. Eine im Programm MARK über die relevanten Modelle gemittelte Populationsschätzung („model average“) ergab eine geschätzte Population von 161 (126 – 252) männlichen und 249 (174 – 495) weiblichen Stücken Rotwild im Untersuchungsgebiet (siehe Kapitel 5). Um zu untersuchen, ob und inwieweit die Annahme der Geschlossenheit der Population verletzt ist, habe ich so genannte Pradel-Modelle, die für offene Populationen geeignet sind, verwendet (siehe Boulanger & McLellan 2001). Dieser Test ergab, dass während des Sammelzeitraums Zu- und/ oder Abwanderung von Rotwild im Gebiet stattgefunden haben können, was bedeutet, dass die Population geografisch nicht geschlossen war. Daher habe ich zur Berechnung der Populationsdichten die von den Transekten abgedeckte Fläche durch einen Puffer von 750 m (entspricht dem Radius eines mittleren Saison-Streifgebiets männlicher Rothirsche) erweitert. Die daraus resultierenden Dichten basieren auf einer Fläche von 129 km² und betragen 1,24 (0,98 – 1,95) männliche und 1,92 (1,35 – 3,84) weibliche Tiere pro km². Das Problem der geografischen Geschlossenheit sollte in zukünftigen Studien im Detail behandelt werden.

Die in der vorliegenden Studie geschätzten Rotwild-Zahlen übertreffen deutlich die bisherigen für das betreffende Gebiet aufgrund von Jagdstrecken oder Scheinwerferzählungen gemachten Annahmen. Der Managementplan für das Rotwild im Wildforschungsgebiet sollte daher überdacht werden.

Teile dieser Arbeit sind in Fachzeitschriften publiziert oder zur Veröffentlichung eingereicht:

Ebert, C., Knauer, F., Storch, I., Hohmann, U. (2010): Individual heterogeneity as a pitfall in population estimates based on non-invasive genetic sampling: a review and recommendations. Wildlife Biology 16, 225-240. [Kapitel 1]
C. Ebert führte die Literaturrecherche durch, analysierte die Daten und schrieb das Manuskript. F. Knauer führte einen Teil der statistischen Bearbeitung durch und hat Korrektur gelesen. Ilse Storch schlug das Thema vor und hat Korrektur gelesen. U. Hohmann half bei der Konzipierung der Recherche und hat Korrektur gelesen.

Ebert, C., Huckschlag, D., Schulz, H.K., Hohmann, U. (2010): Can hair traps sample wild boar (*Sus scrofa*) randomly for the purpose of non-invasive population estimation? European Journal of Wildlife Research 56, 583-590. [Kapitel 2]
C. Ebert hat die Haarbeprobung durchgeführt, die Daten analysiert und das Manuskript geschrieben. D. Huckschlag half bei dem Setup der Videoüberwachungsanlage und des Untersuchungsdesigns. H. Schulz half bei dem Untersuchungsdesign. U. Hohmann trug zum Untersuchungsdesign und zur Themenfindung bei und hat Korrektur gelesen.

Ebert, C., Kolodziej, K., Schikora, T., Schulz, H.K., Hohmann, U. (2009): Is non-invasive genetic population estimation via faeces sampling feasible for abundant mammals with low defecation rates? A pilot study on freeranging wild boar (*Sus scrofa*) in South-West Germany. Acta Silvatica et Lignaria Hungarica 5, 167-177 [Kapitel 3]
C. Ebert organisierte die Kotbeprobung, analysierte die Daten, führte die Modellierung und Populationsschätzung durch und schrieb das Manuskript. K. Kolodziej genotypisierte die Kotproben. T. Schikora entwickelte die Transektanordnung und sammelte die Proben. H. Schulz und U. Hohmann halfen beim Untersuchungsdesign und haben Korrektur gelesen.

Ebert, C., Kolodziej, K., Schulz, H.K., Hohmann, U.: Estimating wild boar (*Sus scrofa* L.) population size using faecal DNA and capture-recapture modelling. Eingereicht bei “Wildlife Biology”. [Kapitel 4]

C. Ebert führte die Kotbeprobung durch, analysierte die Daten, führte die Modellierung und Populationsschötzung durch und schrieb das Manuskript. K. Kolodziej führte die genetischen Analysen durch. H. Schulz half bei der Erstellung des Untersuchungsdesigns und Genotypisierungsprotokolls. U. Hohmann half bei der Erstellung des Untersuchungsdesigns und hat Korrektur gelesen.

Ebert, C., Marell, R., Rahlfs, M., Spielberger, B., Hohmann, U.: Estimating red deer (*Cervus elaphus*) population size based on non-invasive genetic sampling. Eingereicht bei “European Journal of Wildlife Research”. [Kapitel 5]

C. Ebert führte die Kotbeprobung durch, analysierte die Daten, führte die Modellierung und Populationsschötzung durch und schrieb das Manuskript. R. Marell half bei der Erstellung des Genotypisierungsprotokolls und des Genetik-Methodenteils und hat Korrektur gelesen. M. Rahlfs half bei der Entwicklung des Untersuchungsdesigns und der Durchführung und hat Korrektur gelesen. B. Spielberger führte die genetischen Analysen durch. U. Hohmann half bei der Erstellung des Untersuchungsdesigns und der Beprobung und hat Korrektur gelesen.

Die publizierten Artikel wurden mit Erlaubnis der jeweiligen Zeitschriftenverlage abgedruckt.

Chapter 1

Individual heterogeneity as a pitfall in population estimates based on non-invasive genetic sampling – a review and recommendations[1]

Abstract

In recent years much progress has been made in non-invasive genetic methods for various purposes including population estimation. Previous research focused on optimizing laboratory protocols and assessing genotyping errors. However, an important source of bias in population estimates still remains in the field sampling methods. The probability of animals being sampled can vary due to sex, age, social status or home range location. In this paper, relevant literature is reviewed to provide an overview of the occurrence of individual heterogeneity (IH) in the field and how it can be minimised, e.g. by adaptation of sampling design. Thirty-eight articles describing non-invasive population estimation for 12 mammal and two bird species were surveyed. The majority of studies discussed IH as a potential problem. The detectability of IH via goodness-of-fit testing depended on the average capture probability reported in the studies. Field tests for assessing variation in sampling probabilities or validating estimations were carried out in only 11 out of the 38 studies. Results of these tests point out that IH is a widespread problem in non-invasive population estimation which deserves closer attention not only in the development of laboratory protocols, but also concerning the sampled species' characteristics and the field methods. IH can be reduced in the field by carefully adapting the sampling design to the characteristics of the studied population. If this is not reasonable, it may be better to switch to a different sampling strategy.

Keywords: capture-recapture, genotyping, hair sampling, individual heterogeneity, population estimate, faeces, wildlife management

[1] Corresponding publication: Ebert, C., Knauer, F., Storch, I., Hohmann, U. (2010): Individual heterogeneity as a pitfall in population estimates based on non-invasive genetic sampling: a review and recommendations. *Wildlife Biology* 16, 225-240.

Introduction

Reliable estimation of population size remains a major challenge in wildlife research and management. In recent years non-invasive DNA-based population estimation methods have been widely applied in a variety of species. Several standard approaches have been modified to fit genetical implementation, among these are rarefaction (e.g. Frantz et al. 2004) and capture-recapture (e.g. Boulanger & McLellan 2001). In their conventional form, both methods presuppose capture or killing of animals or rely on direct sightings, and are challenged by the possibility of heterogeneous detection probabilities amongst the studied population (Borchers et al. 2002, Petit & Valière 2006). Being most frequently used, capture-recapture (CR) methods are especially vulnerable with respect to individual heterogeneity (Pledger & Efford 1998, Link 2004, Lukacs & Burnham 2005a), i.e. differences between individuals of a population in the probability of being captured (Borchers et al. 2002). Capture- and recapture probabilities may be influenced by age, sex, social status, and individual experience (Baber & Coblentz 1986, Piggott & Taylor 2003). This can generate severe bias in population estimates (White et al. 1982, Minta & Mangel 1989, Sweitzer et al. 2000). Individual heterogeneity (IH) can be accounted for with different modelling approaches (see e.g. Otis et al. 1978, Chao 1987, Chao & Jeng 1992, Pledger & Efford 1998). But the power of goodness-of-fit (GOF) tests and model selection procedures to detect IH in a given data set often is low (Menkens & Anderson 1988, McKelvey & Pearson 2001). Furthermore, as Link (2003 and 2004) has stated recently, IH is far more difficult to model than has previously been recognized, modelling being especially problematic if the causes and extent of IH are unknown. Thus, in order to allow accurate population estimates, IH should either be minimised or quantified as far as possible (Petit & Valière 2006).

Methods based on non-invasive genetic sampling offer solutions for estimation of population size without capturing or killing animals, making them advantageous for rare or endangered species (Kohn et al. 1999, Taberlet et al. 1999, Mills et al. 2000, Piggott & Taylor 2003). It was suggested that the absence of handling can overcome the effects of previous capture history on subsequent catchability, thus certain sources of IH could be reduced (McKelvey & Schwartz 2004, Petit & Valière 2006). The most commonly used non-invasive DNA sources are hairs and faeces for mammals, as well as feathers and faeces for birds (Lukacs & Burnham 2005a). Non-

invasive methods have made CR approaches – which in their conventional form are more suitable for small and abundant mammals – applicable for large, elusive and/ or endangered mammal and bird species (Obbard et al. 2010).

However, despite their advantages, non-invasive genetic methods are also prone to heterogeneity related to biological variability among individuals (Kohn et al. 1999, Wilson et al. 2003, Boulanger et al. 2004b). Moreover, in non-invasive methods IH can interact with bias caused by genotyping errors. Allelic dropout and false alleles can create 'new' false individuals, leading to overestimation in population estimates because recaptures may be concealed, resulting in a decreased recapture rate (Creel et al. 2003, McKelvey & Schwartz 2004). Furthermore, there are some issues in non-invasive genetic CR which are not problematic in conventional CR. In genetic CR the total number of marks in the population is not known and marks may not be unique, because only a subset of each animal's genome is used for identification (Lukacs and Burnham 2005a). Therefore, the danger of misidentification is increased compared to conventional CR. Also, a 'sampling occasion' can be more difficult to define compared to a 'capture occasion', because the moment of the deposition of a sample – e.g. hair or faeces – can not be assessed precisely. This can compromise the concept of population closure (Lukacs & Burnham 2005a). Thus, despite the high potential of non-invasive genetic techniques, there are several issues which can complicate the application of a CR framework for population estimation - in addition to the difficulties already present in the conventional approach.

Until now, great progress has been made in genetic techniques. In particular, much effort has been devoted to quantifying and reducing genotyping errors (Taberlet et al. 1999, Paetkau 2003, Broquet & and Petit 2004, Roon et al. 2005, Miquel 2006). In contrast, fewer attempts have been made for assessing the extent and causes of IH in the field – i.e. due to biological characteristics of the sampled species, to individual attributes or due to sampling procedures (Boulanger et al. 2006). However, information about the causes and the extent of IH is essential to improve sampling designs (Boulanger et al. 2004a). Furthermore, IH in combination with uncertainties caused by genotyping errors can cause multiplicative effects and thus lead to an increase in overall bias. Therefore, it is crucial to address both – IH and genotyping problems – very carefully in order to minimise bias in population estimates.

Based on the recent peer-reviewed literature, this paper aims at

1) providing a survey of the occurrence and treatment of IH in non-invasive population estimation studies, especially with respect to different sampling strategies
2) assessing the impact of sample size and capture probability (p) on the detectability of IH via GOF tests or model selection procedures in CR studies
3) comparing different methods which seem suitable to assess IH in the field – also with respect to the study species and its characteristics

Material and Methods

This review is based on population genetic studies that involve non-invasive sampling for the purpose of population estimation in mammals and birds. We performed a search in Swiss Wildlife Information Service (SWISS) database for peer-reviewed publications using the following search terms: hair trap, non-invasive sampling, genotyping, population estimates, faeces sampling, hair sampling, genetic monitoring. The search yielded 104 titles and was supplemented with published lists of references. In total, we detected 142 articles of which we focused on 38 studies (complete list of references available on request from the corresponding author). We only included papers in which the non-invasive sampling was *de facto* conducted in the field and applied for population estimation; literature reviews and articles dealing with single aspects in the development of sampling methods were excluded. We focused on studies using hair, faeces and feathers as those are the main sources of non-invasive tissue samples. Other sources (e.g. urine, shed skin or buccal swabs) have been much less employed for population estimation until now (Broquet et al. 2007). We also included cases in which a combination of different sampling strategies was applied. We restricted our review to studies using CR or rarefaction (also termed 'accumulation curve' methods; see e.g. Kohn et al. 1999, Eggert et al. 2003) approaches for population estimation, as those are the most commonly used and more prone to be biased by IH compared to e.g. estimation of minimum densities or minimum number alive.

For each study we assessed if IH had been mentioned, i.e. considered as a factor potentially influencing the population estimate. Additionally, we recorded if IH was

detected – e.g. via likelihood ratio tests (program CAPWIRE, Miller et al. 2005), Chi²-tests (program CAPTURE, Otis et al. 1978), via Akaike's Information Criterion (AIC, e.g. in program MARK, White et al. 1999) or GOF testing in program U-CARE (Choquet et al. 2005). Furthermore, IH can be discerned in uneven 'capture frequencies' of sampled individuals (Kohn 1999, Scheppers et al. 2007). The power of tests to detect IH can depend on capture probability (p) (Pollock et al. 1990, Boulanger et al. 2002). Additionally, we suspected the number of sampling occasions and coverage, i.e. proportion of the population sampled, to have an effect on IH detectability. The estimated coverage is significantly correlated with p and was included because not all reviewed studies provided estimates of p. We used logistic regression to evaluate the impact of p, coverage, and sampling occasions on the probability of IH being detected. In this context, we evaluated studies in which IH was detected in the capture frequency or via field test, but not in the GOF or model selection tests as 'not detected'. We included also squared terms of p and coverage since data suggested an optimum somewhere in between of the extreme values. Models were selected based on AIC. For the logistic regression, we used every single population estimate reported in the 38 reviewed articles, since results of several study years or different study areas were often included in one article, resulting in different p and population estimates. Since p and coverage of different analyses reported within the same study could be correlated, we included the studies as random factor. This worked well with coverage but not with p, because for p sample size was low (only 39 of 76 analyses included estimates of p average) and the number of studies reporting only one analysis was high. Therefore, in the case of p we averaged the values for each study and conducted both, a weighted and an unweighted logistic regression without random effects. All analyses were performed using program R (Ihaka and Gentleman 1996). For the mixed effect logistic regression model we used the function lmer of the package lme4 (Bates and Maechler 2010).

For studies using CR methodology, we additionally recorded if IH was included in the estimation model (which is not possible for rarefaction methods). We also searched for studies in which field tests had been carried out parallel to the non-invasive sampling for validation purposes. We put our special attention to methods and results

of these studies and aimed to assess if the applied field tests hold the potential to reveal IH.

Results

The papers we reviewed dealt with 14 different study species, 12 mammals and 2 birds. In 30 of the 38 studies we included in our review, CR was the sole method applied to estimate population size. Four studies used rarefaction analysis only and four used both methods (Table 1). Hair was the DNA source in 22 of 38 studies altogether. In one of these cases, the hair sampling was combined with harvest data and in another, faecal sampling was carried out simultaneously. The remaining 16 studies relied on faeces as DNA source, one of them in combination with feathers. IH bias was mentioned as a potential problem in 34 (89 %) of all contributions, whereas it was modelled in 26 of the 34 capture-recapture studies (i.e. 76 %). In 18 of the 34 capture-recapture articles, IH was detected via Chi^2- tests, likelihood ratio tests or with the help of AIC. A further 5 studies performed GOF tests but did not detect IH in their data sets. In 6 of all 38 studies, tests either failed to detect IH or were not performed, but it was nevertheless visible in the 'capture frequencies' (Table 1). Altogether, in 24 of the 38 studies (63 %) the data revealed the occurrence of IH amongst the studied population independent of further field tests.

Table 1: Overview over all reviewed articles. For each article, every single population estimate is registered separately (e.g. in studies which were carried out over several years or in different study areas). P average is the average per session capture probability reported in the studies (n.r. = not reported). For each study, it is noted if individual heterogeneity (IH) was detected via goodness-of-fit (GOF) testing or model selection procedure.

Reference (study)	Species	Sampling	No. individuals sampled	p average	Estimated N	No. sampling occasions	Software used	IH detected in the field? (test method)	IH detected via GOF (test)
Banks et al. 2003	*Lasiorhinus kreftii*	Hair	81	n.r.	113	7	CAPTURE	-	yes, CF (none)
Mowat & Paetkau 2002	*Martes americana*	Hair	88	0.15	213	4	CAPTURE	-	yes (Chi^2)
Scheppers et al. 2007	*Meles meles*	Hair	55	n.r.	61	1	CAPWIRE	yes (direct sightings)	yes (LR)
Triant et al. 2004 (coast sampling)	*Ursus americanus*	Hair	57	n.r.	77	2	n.r.	-	no (-)
Triant et al. 2004 (inland sampling)		Hair	32	n.r.	41	2	n.r.	-	no (-)
Boersen et al. 2003	*Ursus americanus*	Hair	58	n.r.	119	14	CAPTURE	-	yes (Chi^2)
Immell & Anthony 2007 (Steamboat 2003)	*Ursus americanus*	Hair	32	0,4	46	3	CAPTURE	-	yes (Chi^2)
Immell & Anthony 2007 (Steamboat 2004)		Hair	30	0,2	57	3	CAPTURE	-	no (Chi^2)
Immell & Anthony 2007 (Toketee 2003)		Hair	47	0,3	67	3	CAPTURE	-	yes (Chi^2)
Immell & Anthony 2007 (Toketee 2004)		Hair	46	0,31	65	3	CAPTURE	-	yes (Chi^2)
Belant et al. 2005 (Stockton Island)	*Ursus americanus*	Hair	26	0,68	26	4	CAPTURE	-	no (Chi^2)
Belant et al. 2005 (Sand Island)		Hair	6	0,54	6	4	CAPTURE	-	sample size too small for test
Settlage et al. 2008 (National Park)	*Ursus americanus*	Faeces	129	0,06 to 0,18	97 to 114	10	CAPTURE	-	yes (Chi^2)
Settlage et al. 2008 (National Forest)		Faeces	60	0,09 to 0,32	197 to 330	10	CAPTURE	-	yes (Chi^2)
Poole et al. 2001 (1)	*Ursus arctos*	Hair	98	0,19	148	5	CAPTURE	-	no (Chi^2)
Boulanger & McLellan 2001 (1) (2) (CAPTURE)	*Ursus arctos*	Hair	98	n.r.	155	5	CAPTURE	-	no (Chi^2)
Boulanger & McLellan 2001 (1) (MARK)		Hair	98	0,05 to 0,4	191	5	MARK	-	yes (AIC)

Boulanger et al. 2002 (Jumbo project)	*Ursus arctos*	Hair	33	0,26	45	4	CAPTURE	-	yes (Chi^2)
Boulanger et al. 2002 (U. Columbia 97)		Hair	40	0,2	55	5	CAPTURE	-	yes (Chi^2)
Boulanger et al. 2002 (U. Columbia 98)		Hair	40	0,12	92	5	CAPTURE	-	yes (Chi^2)
Boulanger et al. 2002 (Kingcome)		Hair	58	0,2	102	5	CAPTURE	-	yes (Chi^2)
Boulanger et al. 2002 (U. Columbia 96)		Hair	55	0,16	108	4	CAPTURE	-	yes (Chi^2)
Boulanger et al. 2002 (Granby Kettle)		Hair	22	0,13	46	5	CAPTURE	-	no (Chi^2)
Boulanger et al. 2002 (Prophet)		Hair	98	0,17	166	5	CAPTURE	-	yes (Chi^2)
Boulanger et al. 2004a	*Ursus arctos*	Hair	41	0,35	104	3	MARK	yes (radiotelemetry)	yes (AIC)
Boulanger et al. 2004b (sampling 1996)	*Ursus arctos*	Hair	54	0,16	108	4	CAPTURE	yes (radiotelemetry)	no (AIC)
Boulanger et al. 2004b (sampling 1997)		Hair	41	0,2	55	5	CAPTURE		no (AIC)
Boulanger et al. 2004b (sampling 1998)		Hair	39	0,12	92	5	CAPTURE		no (AIC)
Bolanger et al. 2006	*Ursus arctos*	Hair	41	0,32	43	4	MARK	-	yes (AIC)
Boulanger et al. 2004	*Ursus arctos*	Hair	total: 123	n.a.	per year, mean 49	5	MARK	-	no (-)
Mowat & Strobeck 2000 (British Columbia)	*Ursus arctos*	Hair	109	0,1	257	5	CAPTURE	-	no (Chi^2)
Mowat & Strobeck 2000 (Alberta)		Hair	37	0,16	74	4	CAPTURE	-	yes (Chi^2)
Mowat et al. 2005 (sampling SC Selkirks)	*Ursus arctos*	Hair	38	0,09	97	5	CAPTURE	-	?
Mowat et al. 2005 (sampling NC Selkirks)		Hair	74	0,08	223	5	CAPTURE	-	?
Mowat et al. 2005 (sampling Prophet plateau)		Hair	32	0,13	63	5	CAPTURE	-	no (Chi^2)
Mowat et al. 2005 (sampling Prophet Mtns)		Hair	67	0,21	96	5	CAPTURE	-	no (Chi^2)
Mowat et al. 2005 (sampling Yellowhead)		Hair	48	0,16	107	4	CAPTURE	-	?
Mowat et al. 2005 (sampling Parsnip Plateau)		Hair	21	0,12	50	4	CAPTURE	-	no (Chi^2)
Mowat et al. 2005 (sampling Parsnip Mtns)		Hair	216	0,22	341	4	CAPTURE	-	no (Chi^2)
Mowat et al. 2005 (sampling Parsnip Plateau)	*Ursus americanus*	Hair	194	0,06	892	4	CAPTURE	-	?
Mowat et al. 2005 (sampling Parsnip Mtns)		Hair	85	0,08	363	4	CAPTURE	-	?

Mowat et al. 2005 (sampling Bowron river)	*Ursus arctos*	Hair	53	0,32	76	3	CAPTURE	-	?
Woods et al. 1999	*Ursus arctos, U. americanus*	Hair	54	0.05	104	4	CAPTURE	-	no (-)
Gervasi et al. 2008 (3)	*Ursus arctos marsicanus*	Hair	30	0,03	44	12	MARK	-	no (-)
Frantz et al. 2004	*Meles meles*	Hair	14	n.r.	12 to 19	n.r.	GIMLET/ R (rarefaction)	no (direct counts)	no (-)
Dreher et al. 2007	*Ursus americanus*	hair + harvested bears	544	0,02 (hair), 0,21 (hunt)	1882	5	MARK	radiotelemetry, harvested bears (hair: yes)	no (-)
Wasser et al. 2004	*Ursus arctos, U. americanus*	hair, faeces	24	n.r.	28	5	MARK	radiotelemetry, comparison between sampling methods (no)	no (-)
Cubaynes et al. 2010	*Canis lupus*	Faeces	160	0.01 to 0.86	3 to 126	n.r.	E-SURGE, U-CARE	-	yes (AIC)
Prugh et al. 2005	*Canis latrans*	Faeces	total: 56	0,6 and 0,75	per year, mean 26	9	MARK	radiotelemetry (yes)	yes (AIC, CF)
Prigioni et al. 2006	*Lutra lutra*	Faeces	23	n.r.	36	n.r.	SPSS (rarefaction)	-	yes, CF (none)
Puechmaille & Petit 2007 (sampling Epiniac 2003)	*Rhinolophus hipposideros*	Faeces	54	n.r.	approx. 65	1	R	direct counts (no)	no (LR, S)
Puechmaille & Petit 2007 (sampling Pluherlin 2003)		Faeces	35	n.r.	approx. 42	1	R		no (LR, S)
Puechmaille & Petit 2007 (sampling Saint-Thurial 2003)		Faeces	16	n.r.	approx. 28	1	R		yes (LR, S)
Puechmaille & Petit 2007 (sampling Epiniac 2004)		Faeces	58	n.r.	approx. 85	1	R		yes (LR, S)
Puechmaille & Petit 2007 (sampling Pluherlin 2004)		Faeces	35	n.r.	approx. 48	1	R		yes (LR, S)
Puechmaille & Petit 2007 (sampling Saint-Thurial 2004)		Faeces	14	n.r.	approx. 18	1	R		no (LR, S)
Frantz et al. 2003(4)	*Meles meles*	Faeces	20	0,15	26	10	CAPTURE	-	n.r.
Bellemain et al. 2005 (sampling 2001)	*Ursus arctos*	Faeces	311	n.r.	approx. 480	11	MARK	-	yes (AIC)
Bellemain et al. 2005 (sampling 2002)		Faeces	239	n.r.	approx. 350	13	MARK	-	yes (AIC)
Bellemain et al. 2007	*Ursus arctos*	Faeces	28	n.r.	32 and 47	n.r.	GIMLET/ R (rarefaction)	-	no (-)
Solberg et al. 2006 (sampling 2001)	*Ursus arctos*	Faeces	146	n.r.	223	11	MARK	-	yes (AIC)
Solberg et al. 2006 (sampling 2002)		Faeces	81	n.r.	157	13	MARK	-	yes (AIC)
Jacob et al. 2010	*Tetrao urogallus*	faeces	29	n.r.	78	1	CAPWIRE	-	yes

(sampling Obwalden)									(LR)
Jacob et al. 2010 (sampling Regelstein)		Faeces	9	n.r.	14	1	CAPWIRE	-	yes (LR)
Jacob et al. 2010 (sampling Höhi)		Faeces	16	n.r.	20	1	CAPWIRE	-	no (LR)
Jacob et al. 2010 (sampling Schwägalp)		Faeces	7	n.r.	10	1	CAPWIRE	-	yes (LR)
Jacob et al. 2010 (sampling Rofla)		Faeces	7	n.r.	10	1	CAPWIRE	-	yes (LR)
Jacob et al. 2010 (sampling Salouf)	*Tetrao urogallus*	Faeces	5	n.r.	5	1	CAPWIRE	-	no (LR)
Jacob et al. 2010 (sampling Albula-north)		Faeces	23	n.r.	36	1	CAPWIRE	-	no (LR)
Jacob et al. 2010 (sampling Albula-south)		Faeces	8	n.r.	33	1	CAPWIRE	-	no (LR)
Eggert et al. 2003	*Loxodinta cyclotis*	Faeces	86	0,0 to 0,2	225	10	CAPTURE	-	yes (Chi^2)
Banks et al. 2002	*Vombatus ursinus*	Faeces	17	n.r.	19	5	CAPTURE	-	yes (Chi^2)
Rudnick et al. 2008	*Aquila heliaca*	faeces, feathers	278	> 0,44	308	4	MARK	-	yes, CF (none)
Marucco et al. 2009 [5]	*Canis lupus*	Faeces	total: 87	0,28 to 0,77	per year, 21 to 47	14	MSURGE, UCARE	yes [5] (snow tracking, survey of marking behaviour)	no (AIC)
Meijer et al. 2008	*Alopex lagopus*	Faeces	30	n.r.	42	2	n.r.	-	no (-)
Wilson et al. 2003[4]	*Meles meles*	Faeces	20	n.r.	36	4	Proc NLIN	video control, marked badgers (yes)	yes, CF (none)
Kohn et al. 1999	*Canis latrans*	Faeces	30	n.r.	41	n.r.	JMP IN3	radiotelemetry (yes)	yes, CF (none)

LR = Likelihood ratio test (see Miller et al. 2005) implemented in model selection procedure of program CAPWIRE

S = simulation test

Chi^2 = Chi^2- tests implemented in Program CAPTURE model selection routine (Otis et al. 1978)

AIC = IH suggested by the model selection process (via Akaike's Information Criterion) in program MARK (White & Burnham 1999)

CF = Capture frequency, i.e. IH is visible in the number of times different individuals are sampled

[1] Both articles deal with the same grizzly bear data set (Prophet River Project 1998). In both, Chi^2- tests failed to detect IH. But Boulanger & McLellan (2001) detected IH as a function of distance from study area (via AIC) as well as assumed age-specific capture probabilities.

[2] For Boulanger & McLellan 2001, two different population estimation methods are reported for the same data set. Because of their differing results, both are listed here separately

(3) In this paper, heterogeneity was modelled for each of three different hair sampling approaches applied, but not IH as discussed in our review due to small sample size. Thus, IH was assumed by the authors to be present in the data set, but not detected via tests.

(4) Both articles deal with the same badger data set. In Frantz et al. 2003, results of program CAPTURE's Chi^2- tests are not reported, even though the authors note that they assume the heterogeneity models to be most appropriate. Wilson et al. reported the capture frequencies of individual badgers which revealed IH.

(5) IH was detected, but it did not impact the population estimate

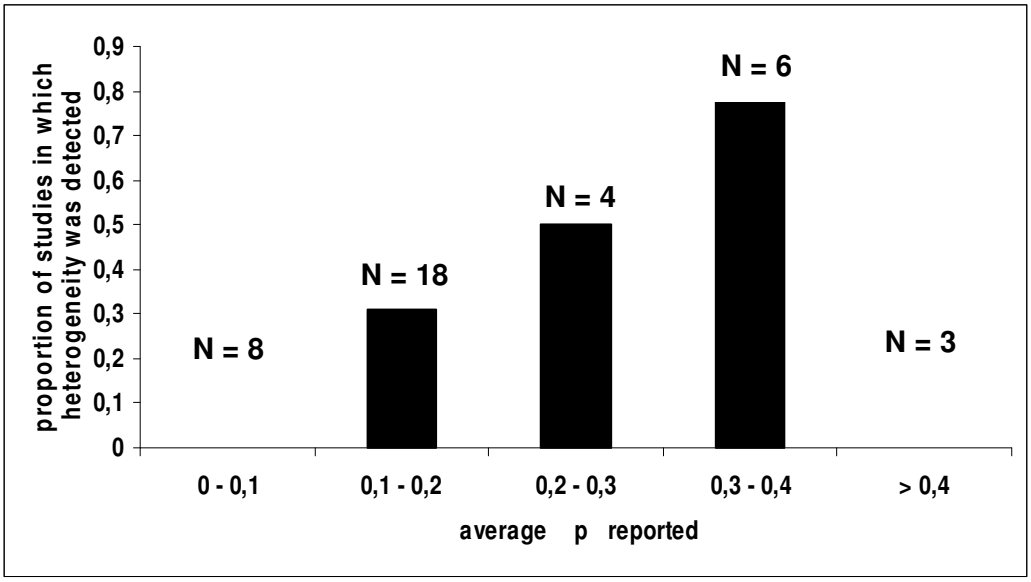

Figure 1: Proportion of population estimates in which individual sampling heterogeneity was detected via goodness-of-fit test in relation to average capture probability (average p reported). Only those studies are included which report an estimate of the average capture probability p (39 of 75 studies, cf. table 1).

ASSESSMENT OF IH VIA GOF TESTS AND MODEL SELECTION PROCEDURES

In this section we are taking into account every single population estimate (N = 76) reported in the 38 reviewed articles. More than half (53.8 %) of the 39 reported estimates of p average lie below 0.2 (Fig. 1) and thus around or below the minimum recommended by Otis et al. (1978) for reliable model selection and population estimates (Otis et al. recommend $p \geq 0.2$ for a population of 200 animals and state that p should never lie below 0.1. Recommended minimum number of capture occasions is 5, better 7 to 10). Furthermore, IH was only detected for p between 0.16 and 0.4. The proportion of population estimates in which IH was detected increased with increasing p until $p = 0.4$ (Fig. 1). In none of 3 studies with $p \geq 0.4$ IH was detected. However, in one of those studies sample size was too small to carry out tests in program CAPTURE (see table 1, Belant et al. 2005, sampling Sand Island). The logistic regression showed no impact of coverage on the detectability of IH (Table 2). In the case of p and p^2, the results suggest that there is an effect on the detectability of IH (Table 3 and 4). IH detectability is highest at p values around 0.3 (Fig 2). The most supported model does not include the number of sampling occasions, but a model including sampling occasions is ranked marginally below ($\Delta AIC < 2$), indicating a potential influence (Burnham and Anderson 1998). It seems

possible that with increasing number of sampling occasions, the detectability of IH increases. Models including an interaction between p and the number of sampling occasions were not supported (table 3).

Table 2: Support of logistic regression models testing the impact of coverage (i.e. the ratio on the detection of individual heterogeneity in the reviewed studies ('cov' = coverage, 'cov2' = squared coverage, 'samp_occ' = number of sampling occasions)

	AIC	Δ AIC	AIC	Δ AIC
Model	weighted		unweighted	
p, p2	27,49	0	14,30	0
p, p2, samp_occ	29,40	1,90	16,12	1,82
p, p2, samp_occ, p* samp_occ, p2* samp_occ	32,64	5,15	19,80	5,50
Null	36,00	8,51	24,08	9,78
Samp_occ	37,45	9,95	25,81	11,51
P	37,45	9,96	24,41	10,11

Table 3: Support of logistic regression models testing the impact of capture probability on the detection of individual heterogeneity in the reviewed studies ('p' = capture probability, 'p2' = squared capture probability, 'samp_occ' = number of sampling occasions).

Model	AIC	Δ AIC
p, p2	27,49	0
p, p2, samp_occ	29,40	1,90
p, p2, samp_occ, p*samp_occ, p2*samp_occ	32,64	5,15
Null	36,00	8,51
samp_occ	37,45	9,95
P	37,45	9,96

ASSESSMENT OF IH VIA FIELD TESTS

Field tests suitable for assessing the occurrence of IH bias were performed in 11 of the 38 studies. In 7 of these 11 cases, IH was actually detected (Table 1). In one of the 4 other cases, IH was found to be present in the hair sampling part of the study, but was strongly reduced by sampling harvested animals as an additional strategy (Dreher et al. 2007). Furthermore, in 8 of the 11 studies, IH was detected via GOF testing or in the 'capture frequencies'. Thus, in two cases where the field tests did not reveal IH, it seemed nevertheless to be present and detectable in the data set. Furthermore, in two cases IH was detected through the field test but not in the data.

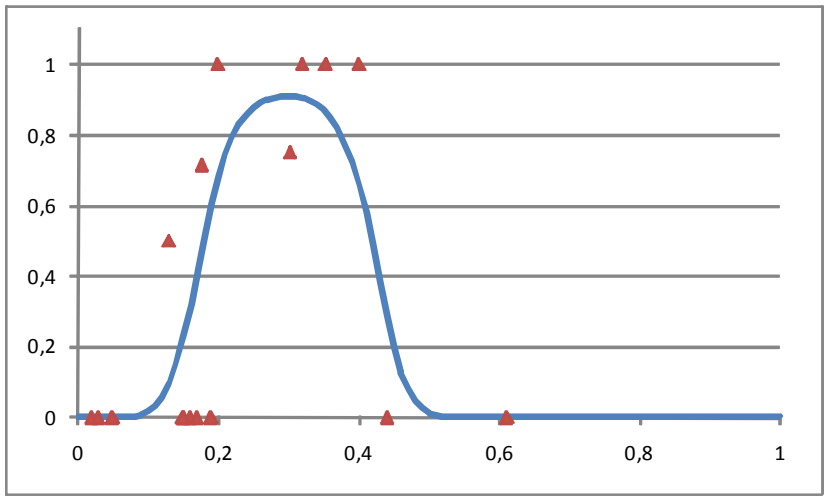

Figure 2: Observed (triangles) and predicted (line) values of IH detectability dependent on p and p2.

FIELD TESTS IN DETAIL

Using radiotelemetry Kohn et al. (1999) found IH to be present in their population under study: 12 radio-collared coyotes (*Canis latrans*) made use of the area to different degrees, and the number of faeces deposited correlated with their relative use of the study area. This IH was also reflected in the 'capture frequencies' of the sampled individuals. Faeces sampling of a coyote population in Central Alaska exhibited IH with respect to age and home range as well as resident status. This was revealed through radiotelemetry of 15 collared adult resident individuals which showed higher survival and recapture rates compared to juveniles and transient or edge individuals (Prugh et al. 2005). Furthermore, the model selection process in program MARK detected IH in the data. This was also the case in a hair sampling study on grizzly bears (*Ursus arctos*): Boulanger et al. (2004a) used location data of 12 GPS-collared bears to evaluate potential bias. They found p to be greater for males than for females and also to be influenced by capture history (i.e. differences between collared and non-collared individuals). The latter was also detected in another study (Boulanger et al. 2004b), in which radiotelemetry was conducted over three years on a total of 35 bears and compared to hair sampling data collected in the same area. Additionally the p of females with cubs differed from those of the rest of the population. In a study carried out by Wasser et al. (2004), grizzly bear faeces sampling data was compared to hair sampling and radiotelemetry data collected simultaneously in the same area. Faeces collection was conducted with the help of trained dogs. This seemed to be an effective and relatively less biased method

compared to hair sampling at baited stations. In the latter, close kin (i.e. females with their offspring) were considerably less represented. However, the sample size of matched faeces and telemetry data was too small to allow more fine-grained comparisons, and IH was neither detected in the field test nor in the data set (Wasser et al. 2004). In a black bear (*Ursus americanus*) hair sampling study accompanied by radiotelemetry, Dreher et al. (2007) used harvested bears as an additional sample. Due to this combination, the IH, which would have been present if hair sampling or harvest data were used alone, was strongly reduced (B. Dreher, pers. comm.) and thus not detected in the field test and in the data set.

Wilson et al. (2003) carried out a faeces sampling study on badgers (*Meles meles*) in which they used video control of a largely marked population to validate their rarefaction estimate. They succeeded in sampling almost the entire population by collecting faeces at latrines near badger setts and did not detect sex- or age class-bias. However, considerable variation existed in the numbers of samples obtained from the different individuals. Moreover some known individuals never used the sampled latrines and thus were not identified via faeces sampling. These results suggest the incidence of IH - e.g. due to variation in individual behaviour - which in that case might not have compromised the estimation, because such a high proportion of the population was actually sampled. A hair sampling study conducted by Scheppers et al. (2007) at badger setts - simultaneously surveyed via direct observations - yielded similar results. The ease with which badgers were sampled varied considerably between setts; hair traps were not visited equally often by all members of the groups. Using baited hair traps and applying direct observation as a validation method, the results of Frantz et al. (2004) show a comparable pattern. Even though obvious variation in the individual sampling frequency existed, almost all badgers present in the area were sampled. Thus, the rarefaction analysis yielded quite reliable results. However, the IH observed in the three badger studies might have been crucial in other populations or situations, e.g. when a lower proportion of the population is represented in the samples, especially when CR methods are applied (Pollock et al. 1990). Furthermore, video control as well as direct observation in all three studies focused on obtaining an independent census of the sampled badger groups, not on observing the sampling behaviour itself. As a consequence potential sources of IH like e.g. dependence of latrine use or access to bait on social

status may remain undetected. The same seems to hold true for a study on the lesser horseshoe bat (*Rhinolophus hipposideros*): the non-invasive population estimate was validated via direct counts of bats in their day roosts (Puechmaille & Petit 2007). The direct counts did not reveal any IH in the faeces sampling. However, IH was detected in the sampling data of several of the sampled bat colonies via likelihood ratio- or simulation tests.

In a faeces sampling study on wolves (*Canis lupus*) in the Italian Alps, Marucco et al. (2009) used an evaluation system for age-dependent marking behaviour related to defecation. Due to the fact that part of the population was radiocollared or otherwise known, it was possible to discriminate between faeces deposited by adult wolves for marking purposes and 'non-marking' faeces. The authors detected age- and status-dependent IH in defecation behaviour and concluded that they would have missed a considerable part of the juvenile population if they had not adapted their search pattern. However, by means of the field tests, Marucco et al. (2009) were able to apply and confirm a representative faeces sampling strategy.

Discussion

Most researchers seem to be aware of IH being a major problem present in population estimation based on non-invasive genetic methods. The vast majority of articles dealing with non-invasive methods applied for such purpose mention or discuss this problem. In most studies based on CR approaches, the authors attempted to account for potential bias by employing models which incorporate IH (Chao 1987). However, as long as the different sources and the extent of IH are unknown, the results of population estimations are strongly model dependent and might not reflect reality (Link 2003, 2004). Furthermore, the different methods to test for IH in the data set may have limited power and thus often fail to detect IH (Boulanger & McLellan 2001, Miller et al. 2005). In the literature, it has been mentioned that the power of such test procedures is especially low for low p (Menkens & Anderson 1988, Boulanger & McLellan 2001). The results of our analysis support this finding: they indicate an impact of p on the detectability of IH via GOF tests and model selection procedures. This effect does not seem to depend on the type of test and the software used (logistic regression with test type as additional covariate showed no significant effect; results not shown). In our analysis, we used a

very conservative approach by averaging over the studies and using both, weighted and unweighted values. Both approaches show very similar results, indicating the results being robust to details in the analysis. In studies with low p, IH was detected considerably less often compared to studies with higher p. The highest proportion of detected IH was attributed to studies with p between 0.2 and below 0.4. Interestingly, in none of the three studies reporting p higher than 0.4, tests suggested the incidence of IH. This might be due to the fact that IH bias becomes much less problematic – perhaps even negligible – when p is high, which has been shown in simulation studies (J. Boulanger, pers. comm.). When most animals in a population are actually captured or sampled, the differences in p between individuals have much less impact on the population estimate (Pollock et al. 1990, Lukacs & Burnham 2005a). Thus, IH might not be reflected in GOF testing or model selection when p is high. Even though the number of sampling occasions for each study was not included in the most supported model, there seems to be an indication for a certain influence on the detectability of IH, because the model including sampling occasions was ranked only marginally inferior to the best model. The more sampling occasions are carried out, the better might be the ability to detect IH via GOF testing. However, this point needs further investigation before a clear conclusion on sampling occasions can be drawn In contrast to p, an impact of coverage on the detectability of IH was not supported in our analysis, despite the correlation of coverage and p.

It should be mentioned that with our analysis we are not able to distinguish if a negative result of testing for IH is due to lack of power and test failure or because there simply is no IH present in a given data set. However, regarding the existing literature including simulation studies and studies on populations of known size, IH seems to be almost ubiquitous in non-invasive sampling data sets like in conventional CR (Pollock et al. 1990, Borchers et al. 2002, Knapp et al. 2007, Lukacs & Burnham 2005a). Therefore, it seems much more likely that a negative test result is caused by low test power than that a data set is really homogeneous, particularly when p is low. In recent years, new modelling approaches, e.g. multistate and multievent models (Pradel 2005), have been developed which might allow a more flexible handling of CR data in presence of IH (Crespin et al. 2008). In this context GOF testing using nonparametric methods, like in program U-CARE, seems to be quite promising compared to conventional methods (Choquet et al. 2009, Cubaynes et al. 2010).

In 5 of the articles reviewed here, IH was not detected by the data tests which were performed. However, in 2 of the 5 cases, additional field tests were carried out, and both of them revealed IH. In general, data tests and/or pronounced differences in the 'capture frequencies' indicate presence of IH bias without carrying out extra field tests, but often further investigations would be required to uncover the causes of IH. Since many different IH sources exist, they can influence estimations in different ways and this effect may also depend on the sampling design (Crespin et al. 2008). Models that are relatively robust to IH generally show reduced precision of estimate (Boulanger 2004b). This may not be tolerable in cases where an accurate estimate is particularly important, e.g. when the spread of diseases is concerned (Artois et al. 2002) or when management plans for rare or endangered species are considered (Guschanski et al. 2009). However, for endangered species, overestimating a population is much more critical than underestimating it (Meijer et al. 2008), so some underestimation bias may be tolerable in certain cases.

ASSESSMENT OF IH VIA FIELD TESTS

The choice of methods to test for IH in the field strongly depends on the observed species and its behavioural patterns as well as its space and habitat use. Thus, e.g. for badgers, which live in social groups, share setts and make rather small-scale movements, video control or direct observations at setts seem to be an adequate method to validate non-invasively obtained estimates (see e.g. Frantz et al. 2004; Scheppers et al. 2007). Contrastingly, for highly mobile species like bears and also coyotes, radiotelemetry may be more promising. The suitability of a field method to test for IH furthermore depends on the applied sampling strategy. For example, video control or direct observations can be appropriate for surveillance of discrete sampling stations like hair traps or badger setts, but will not be suitable for large-scale sampling designs like e.g. line transects. Radiotelemetry may be more effective to observe movements and transect- or trap-encounter rates of animals on a large scale. Furthermore, radiotelemetry is useful for obtaining information on spatial distribution and home range sizes of animals in order to fit sampling designs and to account for closure violations and edge effects (Boulanger et al. 2004b, Dreher et al. 2007). The feasibility of a sampling method for a given species or population can depend on spatial characteristics like home range sizes and distribution of animals in

the sampled area. Settlage et al. (2008) found hair sampling of black bears via baited sampling stations impractical for the Southern Appalachian region: due to small home range sizes of the resident bears, sampling probabilities were low and biased. In order to yield a reliable estimate, a much higher sampling intensity would have been necessary (Settlage et al. 2008). Grizzly bears showed considerably higher p with comparable sampling intensities because of their larger average home ranges (Boulanger et al. 2004b, McLoughlin et al. 2003).

INTERACTIONS BETWEEN SAMPLING STRATEGIES AND STUDY SPECIES' CHARACTERISTICS

The occurrence and/ or extent of IH may differ dependent on the applied sampling method. 'Active' sampling methods, like hair sampling via baited hair traps, presuppose that animals actively approach the sampling station. In many species, it has been shown that individuals show consistent or context-specific personality traits, e.g. they differ in their exploration behaviour and their reactions towards newly introduced factors, which may affect their sampling probability (Coleman and Wilson 1996, Ruis et al. 2000, Dingemanse et al. 2003, Mettke-Hofmann et al. 2005). Furthermore individual experience and life history may influence behaviour with respect to sampling stations. This could cause IH which is not necessarily related to sex, age or social status, and which might be hard to quantify and very difficult to account for in a model. Thus, in some cases, it may be reasonable to apply a different sampling method. In this context, 'passive' sampling strategies like e.g. faeces sampling along transects represent an alternative which may be less affected by individual behaviour or status differences. This may hold true particularly for group living species: interactions between animals can increase IH, especially when sampling concentrates on defined stations like e.g. hair traps which require active approach. As an example, we conducted a hair sampling pilot study on wild boar (*Sus scrofa*). Video observation at baited hair traps revealed significant behavioural differences depending on age of the animals and on their group status (Ebert et al. 2010). However, also for bears which can be considered as living mainly solitary, it has been shown that via faeces sampling – a 'passive' sampling strategy – a larger part of a population can be observed compared to hair sampling as an 'active' approach (Wasser et al. 2004). Wasser et al. applied both methods in the same study area and time period, and via hair sampling, only 46% of the individuals that were identified via faeces sampling were detected. 'Passive' sampling methods in most

cases will not yield completely unbiased results (in fact, most of the faeces sampling studies reviewed here reported IH in their data sets). Nevertheless, 'passive' sampling might rule out certain sources of IH which are not avoidable in 'active' approaches and thus holds the potential to yield results with smaller overall bias. However, in some (especially social and/ or territorial) species, status or age differences between individuals may cause differences in faeces deposition patterns, leading to IH in detection probabilities. This has been shown e.g. for wolves (Marucco et al. 2009, Cubaynes et al. 2010). Thus, 'passive' sampling will not be suitable in all cases, and at any rate the appropriate sampling strategy and design have to be carefully tested for each particular species and population. Furthermore, the DNA quality of faeces in some cases has been shown to be inferior to that of hair, thus population estimates derived from faeces sampling data may be more in danger of bias due to genotyping errors (Piggott and Taylor 2003).

RECOMMENDATIONS

Perform a pilot study - not only in the lab, but also in the field: In any case, it is most advisable that researchers who plan to establish population estimation based on non-invasive genetic sampling perform pilot studies not only to assess genotyping error rates, but also to detect sources of IH bias in the field. The fact that the majority of reviewed studies in which such field tests were performed actually detected IH highly supports this recommendation. The appropriate methods to assess IH in the field depend on the species or population under study as well as on the applied sampling method.

Do not rely solely on GOF testing and model selection procedures: This holds especially true when p and coverage are low! It can be reasonable to incorporate heterogeneity in an estimation model even if tests suggest that there is no IH present in a data set, because their power is often low. It is always recommended to include biological knowledge and information about study species, habitat etc. to validate model choice.

Try to reduce IH by adapting sampling design: Knowledge about the sources and extent of IH can enable researchers to adapt the sampling design to account for the bias. Among the methods to reduce IH bias in the field, the application of two or more

different sampling strategies in combination seems especially promising (Dreher et al. 2007, Boulanger et al. 2008, Settlage et al. 2008). If multiple methods are used simultaneously to sample a population, the impact of IH caused by any single method can be minimised (Pollock et al. 1990, Williams et al. 2002). The improvement of estimations based on multiple approaches increases with decreasing correlation between the applied sampling methods (Boulanger et al. 2008). In the case of hair sampling, the use of unbaited sampling stations (e.g. installed at trails or rubbing trees) and changing of sampling locations between sessions may be applied to reduce IH due to competition between individuals for resources and due to "trap happy" individuals (Scheppers et al. 2007, Boulanger et al. 2008). Collection of faeces samples with the help of trained dogs seemed to increase detection rate and efficiency of the method considerably, allowing a relatively representative and unbiased population survey compared e.g. to hair sampling (Wasser et al. 2004, Long et al. 2007). Furthermore, it is advisable to perform a sufficiently high number of sampling occasions in order to increase the overall sampling probability and thus to facilitate accurate estimates.

Try to sample a large part of the population: As shown e.g. in the three badger studies reviewed here, one effective way to reduce bias caused by IH is to sample a large proportion of the population (Lukacs & Burnham 2005a). This is generally desirable and has been recommended in relevant literature many times before (see e.g. Otis et al. 1978, Pollock et al. 1990), but is certainly not always feasible. Furthermore, an increase in sample size can have an unfavourable impact on non-invasive genetic population estimates: the more samples are analysed, the higher the misidentification rate due to genotyping errors (McKelvey & Schwartz). Thus, careful error-checking protocols for genotyping are crucial and genotyping error rates should be determined in order to avoid an increase in bias through misidentification (Maudet et al. 2004, Roon et al. 2005).

Consider switching to other sampling strategies: Adapting the sampling design may not always be possible or may yield no success. Furthermore, an unsolved problem still remains: even though detection of IH and its sources may be possible with methods like e.g. radiotelemetry or video observation, the exact quantification of such variation and thus its incorporation in estimation models still seems to be very

difficult. Consequently, when reduction and/ or modelling of IH is not possible, it can be recommendable to apply a different sampling method in some cases. The suitability of a method can depend e.g. on characteristics of the studied species, population, or study area. In some cases, 'passive' sampling approaches may yield more representative results compared to 'active' methods. In case IH can not be reduced or avoided, a study should be designed in a way that it results in capture probabilities between 0.2 and 0.4 to have an ample chance to detect existing IH.

In conjunction with problems caused by genotyping errors, IH is a highly challenging issue in non-invasive population estimation. It is a well-known and explicitly discussed problem at least with regard to its theoretical and model-based aspects. IH can be identified and strongly reduced, when field sampling design and analytical approach are carefully prepared. However, more attention should be given to the evaluation of field methods to bring forward more effective and sustainable population estimates, which is especially important for conservation of endangered species and even more in fragmented habitats.

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Chapter 2

Can hair traps sample wild boar (*Sus scrofa*) randomly for the purpose of non-invasive population estimation?[2]

Abstract

Reliable estimation of population size remains a major challenge in wildlife ecology and management. Lately, genotyping of non-invasively obtained tissue samples integrated in a modified capture-recapture approach provides new perspectives. Faeces, moulted feathers, or hairs can be easily sampled in the field. However, an important assumption is homogeneity of sampling across the population. In this pilot study, we tested the suitability of baited barbed wire hair sampling stations ('hair traps') for homogeneous genetic sampling for population estimation. A video system based on a new network internet protocol was used to observe the behaviour of wild boar visiting baited hair traps for gaining information about potential heterogeneities in the individual sampling probability. Within 92 monitoring nights at two sampling stations, 216 wild boar visits were recorded and 142 hair samples containing 2124 single hairs were collected. Video analysis revealed distinct differences in the behaviour of wild boar with respect to the sampling station which are most likely to result in heterogeneous individual sampling probabilities. Adult and subadult animals differed in their behaviour dependent on their group status. This result indicates that hair sampling with baited hair traps is not suitable for representative non-invasive sampling of free ranging wild boar populations.

Keywords: capture-mark-recapture, individual heterogeneity, population estimate, video control, wildlife management

Introduction

Since 1980, continuously increasing hunting bags (Sàez-Royuela & Telleria 1986, Melis et al. 2006) suggest increasing wild boar population densities in many parts of Europe (Kaden 1998). Advancing agricultural damages and the immigration of wild

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boar into urban areas cause ecological and epidemiological concerns (Maillard et al. 1996, Vassant 1996, Schnidig-Petrig & Koller 2004) including the risk of transmission of Classical Swine Fever (CSF) into domestic pig populations (Hromas 1996, Kaden 1998, 1999, Artois et al. 2002).

Therefore, an effective wild boar population management becomes increasingly important (Truvé 2004). Reliable estimates of population size are highly desirable, e.g. for harvest planning and for monitoring effectiveness of population control (Sweitzer et al. 2000, Miller et al. 2005). Wild boar are difficult to survey because of their complex social structure, nocturnal activity pattern and preference of dense vegetation (Briedermann 1990, Cahill et al. 2003). Traditional approaches to population estimation of wild boar and other ungulates include hunting bag analysis (Boitani et al. 1995a, Acevedo et al 2007), direct sightings (Groot Bruinderink & Hazebroek 1995), and counts of faeces (Vicente et al 2004). These methods may indicate population trends, but cannot reveal absolute population numbers (Boitani et al. 1995, Baubet 1998, Monaco et al. 2004), which are desirable for epidemiological reasons, especially with regard to CSF (Artois et al. 2002). One method which seems promising in this respect is the Capture-Mark-Recapture (CMR) approach (Otis et al. 1978).

For wild boar, capture and recapture probabilities may vary greatly between individuals, being influenced by age, sex, social status, and individual experiences (Baber & Coblentz 1986, Briedermann 1990). This violates one of the main assumptions of mark-recapture modelling, which requires equal capture probability for each individual of the population (White et al. 1982, Minta and Mangel 1989, Sweitzer et al. 2000). The use of photo cameras or sightings instead of recaptures may help to reduce bias (Wild boar: Sweitzer et al. 2000, Fattebert et al. 2004, Hebeisen et al. 2008; roe deer: Focardi et al. 2002), but this so-called “mark-resight method” remains labour-intensive and still requires one initial capture period to mark individuals (Foran et al. 1997).

In recent years, methods based on non-invasive genetic sampling offer solutions for the estimation of population size without capturing animals (Taberlet et al.1999, Beja-Pereira et al. 2009). This approach could reduce individual heterogeneities and thus

result in a more representative survey. Among the possible tissue sources for genotyping are hair and faeces. Hair sampling via hair traps has been used in population estimation studies e.g. for carnivores with most of these studies being based on CMR-modelling (Lukacs & Burnham 2005a, Mulders et al. 2007, Boulanger et al. 2008, Beja-Pereira et al. 2009). To evaluate the reliability of a population estimate based on non-invasive sampling, it is necessary to assess the heterogeneities in sampling probability for the relevant species and sampling procedure (Minta & Mangel 1989, Bellemain et al. 2005, Petit & Valière 2005, Fickel & Hohmann 2006).

In this study, the potential of hair traps made of barbed wire to obtain wild boar hair samples non-invasively is tested. To address the question of sampling representativeness, especially with respect to heterogeneities in the individual sampling probabilities, hair traps were monitored with the help of a remote video system to document wild boar behaviour. The intention was to allow for a first evaluation of the feasibility of non-invasive hair sampling without having to carry out cost- and labour-intensive genotyping of hair samples.

The aims of the study were:

1) To test the hypothesis that the amount of hair snared to the hair trap should increase with the number of wild boar visiting the hair trap and with the number of times the barbed wire was crossed by the animals.
2) To observe the behaviour of wild boar around hair traps and determine whether gender, age and group size could bias sampling.

Material and Methods

STUDY AREA

All experiments were carried out in a site of 4000 ha situated in the Palatinate Forest in south western 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. The predominant native plant community is beech forest (Luzulo-Fagetum). The area is covered with forest to approximately 90% (50% *Pinus* sp., 20% *Fagus sylvatica*, 11% *Picea abies*, 8% *Quercus petraea* and *Quercus robur*). 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)

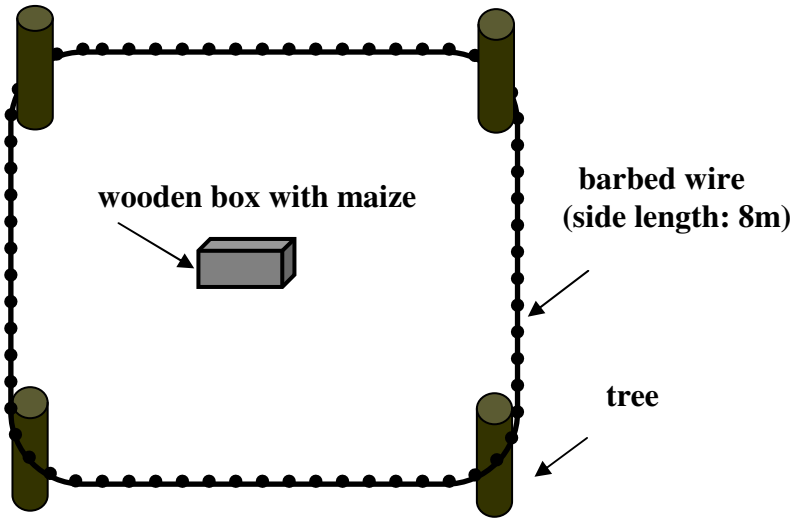

Figure 1: Scheme of a hair trap. The barbed wire is stretched in a height of 30 – 40 cm around 4 suitable trees with a total length of approximately 32m. To get access to the bait, wild boar have to pass underneath the wire. The wire barbs serve as hair snaring device.

HAIR SAMPLING

To obtain hair samples, two sampling stations ('hair traps') were installed in the study area in March 2006 in a distance of 2.75 km to each other. Both stations were situated in mixed forest. We chose locations with signs of wild boar presence (rooting, tracks) in the nearer area but we avoided setting up hair traps near obvious wild boar trails, since trails traversing a hair trap could influence the wire crossing behaviour of visiting boar. Each station consisted of approximately 32 m of barbed wire stretched between 4 suitable trees in a height of approx. 30 – 40 cm above the ground to form a square with a side length of ca. 8 m (Fig. 1). The stations were baited daily with maize offered in a wooden box to prevent non-target species from consuming it. In order to determine if this 'centralized' mode of baiting influenced the

behaviour of the visiting wild boar, it was changed after the first 51 monitoring nights: the maize was then offered in 5 to 6 shares distributed throughout the hair trap ('decentralized'). To reach the bait, for both baiting modes wild boar had to pass beneath the barbed wire, the barbs serving as a hair snaring device. The sampling stations were monitored via video control in the period May to August and June to August 2006 respectively (Table 1).

Both stations were checked daily, all hair snared to the hair traps was collected. The location of each hair sample on the barbed wire was recorded (for later analysis, the 4 sides of the square were referred to as A, B, C, and D, and the wire barbs were consecutively numbered). A hair sample was defined as the hair snared to one wire barb after one night of observation. Furthermore, the absolute quantity of hair snared to each barb was determined by counting the number of single hairs in each sample.

VIDEO CONTROL

A Mobotix- M10 digital network IP- video camera (Mobotix AG Security -Vision-Systems, Germany) was used for monitoring. It was installed in a tree approximately 2.5 to 4 m above the ground (depending on the location and the size of the area to be monitored). For technical details concerning the camera system and its installation see Huckschlag (2008). The monitoring area was illuminated with infrared spot lights (Model 84/30-880, Uniserve Company, Germany). Three to four spot lights were necessary to sufficiently illuminate a the four sides of a hair trap, an equal level of illumination of the four sides of the hair trap being important to record the behaviour of the visiting wild boar properly. For viewing the stored video data, the accessory software package MOBOTIX MxPEGViewer Version 1.1.9. was used (detailed description in Huckschlag 2008).

DATA ANALYSIS

For each videotaped wild boar visit, the following parameters were recorded: Date and time of the visit, number and age of visitors (classified by their size: large individuals as adults, intermediate individuals as subadults and small individuals as piglets). For piglets and subadults, video observation does not allow a reliable discrimination between males and females. Therefore, all piglet and subadult visitors were classified only according to their age, and it was recorded if they arrived as part

of a group or alone. Additionally, for adult wild boar, gender was determined visually whenever possible based on primary and secondary sexual characteristics. Between different visits in the same night or in consecutive nights a definite discrimination of individuals was not possible. Consequently, for comparing visits the number of visitors was subjected to analysis rather than the number of individuals. Within one single visit, discrimination between the individual visitors was possible primarily due to differences in size. Piglets which were still striped were excluded from the analysis, being too small to contact the wire when crossing it and leaving hair samples behind. Therefore, the effective maximum group size for hair sampling analysis included only subadult and adult individuals. For hair trap data analysis, we additionally counted how often each wild boar crossed the barbed wire for each of the four sides (A, B, C, D) separately. The number and location of hair samples on the wire were also recorded for comparing with the locations of the observed wire crossings.

STATISTICAL ANALYSIS

We did not assume a normal distribution for individual crossing frequencies as well as for number of hair samples and hair quantity. Thus, Kruskal-Wallis and Mann-Whitney U-tests were used for comparing crossing frequencies between the age classes and between group and single visitors as well as for comparing the crossing frequency to the quantity of hair snared on the wire. Data from both hair traps were pooled for comparison. The relationship between the number of crossings and the number of hair samples obtained was tested using a Spearman's rank correlation. All analyses were performed using SPSS 14.0 (SPSS Inc., 1989 – 2005).

Table 1: Overview of the video observation data of 2 baited hair sampling stations (hair traps).

	Hair trap 1	Hair trap 2
Period of video control	20.4.2006 – 09.8.2006	26.6.2006 – 10.8.2006
Number of monitoring nights with wild boar visits	60	35
Number of visits during the monitoring nights	163	53
Number of monitoring nights with hair samples	34	13
Number of hair samples during monitoring period	124	18
Number of observed wire crossings	486	128
Ratio of wire crossings to hair samples	3.92 to 1	7.11 to 1
Total number of single hairs collected during monitoring	2073	51

Results

Between 20.4.2006 and 10.8.2006, 216 visits of wild boar at 2 different hair traps were recorded in 95 nights. In 47 of these nights, hair samples from a total of 142 wire barbs were collected the next mornings (Table 1). In 57 of the 216 visits, the visitor was a single wild boar, in the other 159 visits, two or more individuals were observed. Mean visitor group size was 2.03 (SD = 0.83, Range 1 – 4; piglets excluded) animals. In hair trap 1, the ratio of the total number of wire crossings observed during the sampling period to the number of hair samples collected was 3.91:1, so approximately every fourth crossing by a wild boar resulted in leaving a hair sample. In hair trap 2, approximately every seventh crossing resulted in hair being snared (Table 1).

We found no relationship between the number of wild boar visiting a hair trap per night and the quantity of hair snared to the hair trap in the following morning (Spearman's rank correlation, $r_s = 0.284$, p 0.231, n = 41).

During the 216 visits, a total of 430 adult and subadult wild boar visitors were observed. We compared the behaviour of these visitors with respect to their age class and to the fact weather they arrived alone or as part of a group (Fig. 2): subadult wild boar visiting a hair trap in a group crossed the wire more often than

subadults arriving alone (U-test, Z = -2.360, p = 0.018, N = 344). The same holds true for adult animals (U-test, Z = -5.442, p < 0.0001, N = 86). When comparing between the two age classes, adult wild boar arriving as members of a group crossed the wire more frequently than juvenile group visitors (U-test, Z = -2.289, p = 0.022, N = 371). In contrast to this, when arriving alone, adults crossed the wire much less often than juveniles (U-test, Z = -3.623, p < 0.0001, N = 59). In fact, in 16 of 23 (69.6%) of all visits of single adult wild boar, the animals did not cross the wire at all but stayed outside the hair trap.

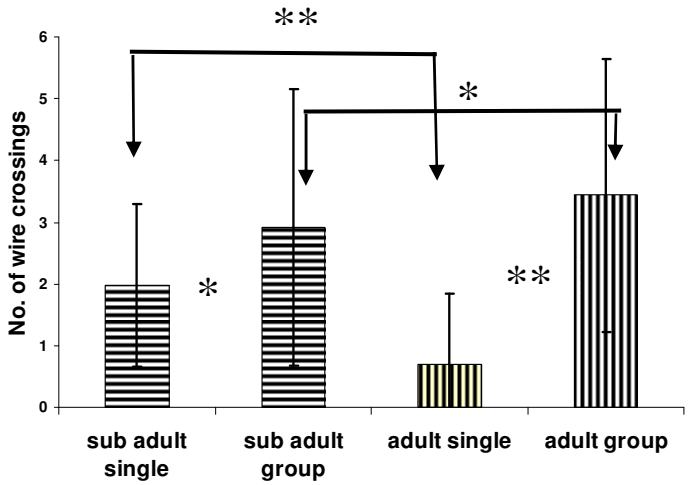

Figure 2: Wire crossing behaviour of adult and subadult wild boar as observed visiting a hair trap. The number of crossings is shown for animals visiting hair traps as part of a group and for single visitors of each of the two observed age classes separately. Significant differences among and between age classes dependent on group status of visits are marked with asterisks (U-tests, * p < 0.05, ** p < 0.01), for details see text.

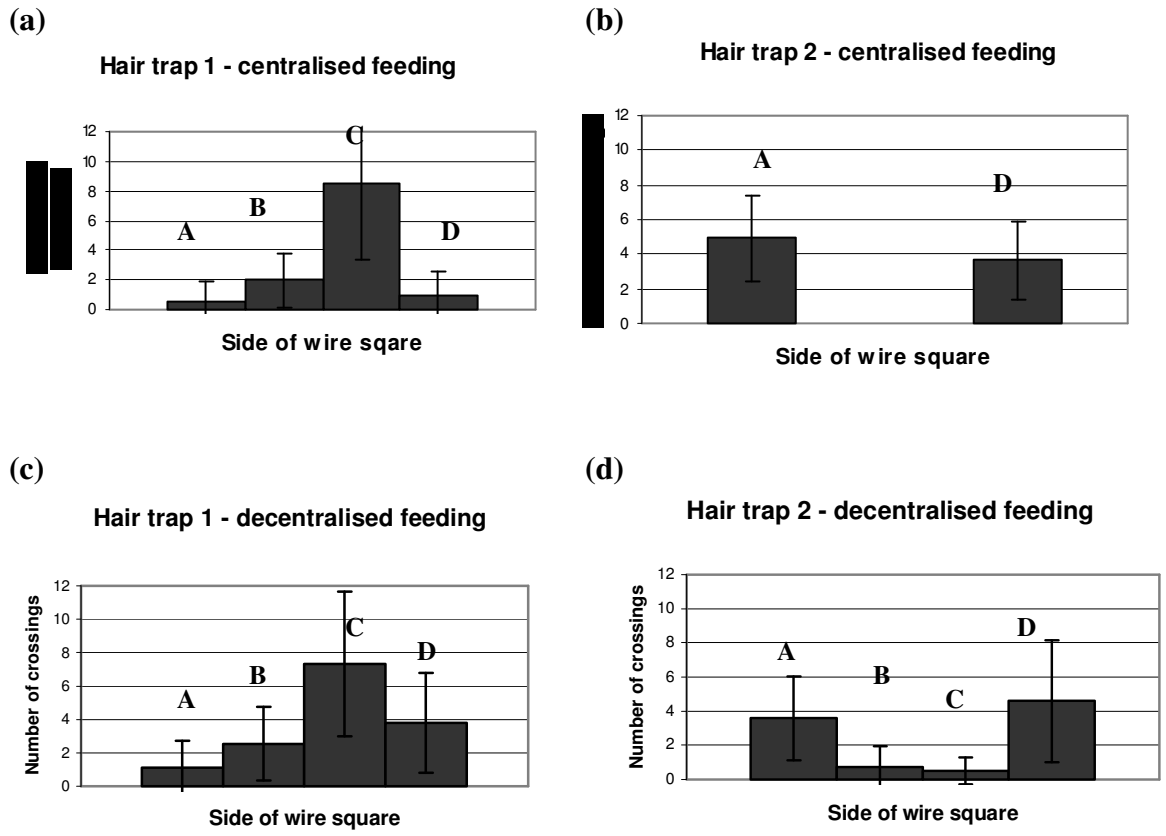

Figure 3: Degree of utilisation for wild boar crossing as related to hair trap side (A, B, C and D indicate the four sides of the wire square). (a) and (b) show the results for visits observed during centralised baiting at hair trap 1 and 2 respectively; (c) and (d) show the results for visits observed during decentralised baiting at hair trap 1 and 2 respectively.

A comparison of the crossing frequencies between the four sides of the wire square shows an accumulation of crossings in both hair traps for the 'centralized' baiting mode (Figs. 3a and 3b). The wild boar seemingly preferred certain sections of the hair traps for crossing. Corresponding to this, in hair trap 1 significantly more hair samples were collected from the most frequented side C than from the other three sides (Kruskal-Wallis test: $\chi^2 = 53.48$, $p < 0.001$, N = 31 monitoring nights). In hair trap 2, only two sides (A and D) were used for crossing, but approximately to the same degree (Fig. 3c). There was no significant difference between side A and D in the number of hair samples collected (Mann-Whitney-U test: $Z = -1.822$, $p = 0.068$, N = 32 monitoring nights). The change in baiting mode ('decentralized') after 51 monitoring nights did not result in a significant change in the crossing behaviour of any of the visitors observed at hair trap 1 and 2 (hair trap 1: Kruskal-Wallis test: $\chi^2 = 4.0$, p 0.135; hair trap 2: Mann-Whitney-U test: $Z = -0.218$, $p = 0.828$; both N = 51

monitoring nights; Fig. 3c and 3d), even though the distribution of crossings was slightly less clumped compared to the 'centralized' baiting mode.

Furthermore, the number of hair samples was not correlated to the number of crossings per night (Spearman's rank correlation, $r_s = 0.180$, $p = 0.188$, $n = 47$). This result is probably caused by the fact that the crossing behaviour concentrates on narrow sections of the hair catcher. However, the number of crossings was correlated to the total number of hairs snared to the wire on the sections crossed by the visitors: the more frequently a wire section was used, the greater the quantity of hair snared to the corresponding section (Spearman's rank correlation, $r_s = 0.511$, $p < 0.01$, $n = 47$). Thus, hair accumulated on the most frequently crossed wire sections.

Discussion

The video observation of hair traps and the sampling results showed that the hair snaring procedure works on principle, although the quantity of hair is rather low compared to the number of wire crossings. As a consequence, the efficiency of the hair snaring mechanism still should be improved, e.g. by altering tightness or height of the barbed wire. The heterogeneity in the behaviour of wild boar visiting the hair traps seems to be related to their age and experience as well as to their group status. An indication of the former is that the adult females - when visiting the hair trap as part of a group - crossed the wire more often than their offspring and in general were more reluctant to remain inside the hair trap and to feed on the bait. In contrast to this, adult wild boar behaved differently when visiting a hair trap alone, crossing the wire much less often or not entering the hair trap at all. This corresponds to the behaviour of adult females observed at live traps during capture attempts, where they mostly stayed in front of the trap without entering (Baubet 1998, C. Ebert, unpublished data, Saebel & Keuling, pers. comm.). In all 159 visits of wild boar groups, the video observation showed that individuals interacted with each other while visiting a hair trap, subdominant wild boar being chased by dominant group members. Thus, hierarchic behaviour also seems to contribute to the heterogeneities in individual crossing behaviour. The differences in crossing behaviour of subadult as well as adult wild boar support this observation: animals arriving in a group crossed the wire significantly more often than those arriving alone. This indicates that interactions between individuals do have an influence on the crossing behaviour. We assumed that interactions might depend on how the bait was offered. Thus, we

aimed to reduce the impact of hierarchic behaviour by offering bait in multiple shares distributed inside the hair traps, allowing several animals to feed on the bait simultaneously. However, this did not result in a behavioural change. In any case, the observed heterogeneity in individual wire crossing behaviour will most probably result in an increased heterogeneity in individual sampling probability, because wild boar which have crossed the wire more often than others are more likely to be represented in the hair samples. Wild boar groups will most probably be over-represented in the survey compared to single animals. In wild boar, females mostly live in family groups and subadult as well as adult males live mainly solitarily (Briedermann 1990). Therefore, a sex bias in sampling probability is very likely. One possibility to account for this problem is to consider only females in later analysis. However, in this case a monitoring of whole wild boar populations would not be feasible. Since family groups dominated by females are the main subject of regulatory management measures, this might not be problematic for many concerns (Keuling et al. 2008).

The preference of certain sections of the hair trap resulting in an accumulation of hair could be related to the course of trails habitually used by the wild boar and leading through the hair catcher. Even though we tried to avoid installing hair traps upon wild boar trails, we can not exclude this possibility, because trails are not always clearly visible and may have been overlooked. If so, this problem might be inherent to our method and very difficult to solve. The observed accumulation of hair at certain wire sections could have different consequences: on the one hand, a wire barb could be “saturated” with hair after several animals crossed at the same section, resulting in an under-representation of subsequently crossing individuals. The fact that more frequent crossings did not result in more wire barbs with hair but in an accumulation of hair on few barbs could be an indication for a certain “saturation effect”. On the other hand, the later crossings of wild boar could rip out hair left by the first visitors. In both cases, the capture probability of the individuals having crossed the wire will most probably be biased. Furthermore, the difficulty of obtaining a representative sub-sample of the hair snared on the wire will be increased (Creel et al. 2003). However, the analysis of all the collected hair will not be feasible in most cases of hair sampling. Thus, sub-sampling often is necessary to keep cost and effort for genotyping hair samples in the laboratory feasible (Sloane et al. 2000) and to minimize the risk of obtaining false genotypes originating from more than one

individual (Frantz et al. 2004). Single hairs should be taken, making certain that only one animal is sampled at a time. Fickel & Hohmann (2006) showed that for wild boar, single hairs can yield sufficient amounts of DNA for genotyping. Sub-sampling is difficult because often more than one visitor crossed the wire at the same hair trap section. By taking e.g. only one hair per wire barb for analysis, one might under-represent animals having visited the hair trap and crossed the wire less often than others. On the other hand, analysing too many single hairs will increase the cost and the risk of analysing multiple samples of animals which crossed the wire more often than others.

The finding that the quantity of hair snared to the hair traps is not correlated to the number of wild boar having visited it in the night before, reflects the heterogeneities observed in the behaviour of the visitors. The quantity of hair and thus the sampling success is only correlated to the number of wire crossings, which has been shown to differ between individuals depending on their age and group status. It may be deduced from this that the hair sampling procedure presented here is not useful for representing the collective of wild boar which actually visit a hair trap and even more it will most probably fail to allow a representative survey of wild boar populations. As a conclusion, the hair sampling method investigated here does not seem suitable for application in population estimation of wild boar, even though the mechanism of hair snaring worked on principle. However, it might be useful for purposes other than population estimation. Furthermore, it might be worthwhile testing other hair sampling mechanisms for wild boar: For example, a device which allows sampling only one single individual at a time may help to reduce heterogeneity, as has been developed for black bears (Immel & Anthony 2008). The sampling procedure might also be improved by using two strands of barbed wire stretched in different heights to facilitate sampling of wild boar of different sizes and ages (see e.g. Boulanger et al. 2006 for grizzly bears). Furthermore, it seems possible that using a non-baited sampling system, e.g. with one-section wire strands at wild boar trails, could reduce the impact of group interaction behaviour on sampling success.

Non-invasive hair sampling methods have been applied successfully on several carnivore species (Foran et al. 1997, Woods et al. 1999, Mowat & Strobeck 2000, Mowat & Paetkau 2002, Mowat et al. 2005). In contrast to brown bears (*Ursus*

arctos), lynxes (e.g. *Lynx lynx, Lynx rufus*) and other carnivores that live mainly solitarily, the wild boar is a social species in which at least the females and their offspring occur in groups of up to 30 individuals and with a certain hierarchy (Briedermann 1990, Kaminski et al. 2005). Thus, the behaviour of wild boar visiting a hair trap as members of a group is most probably influenced by the behaviour of the other group members in addition to the variability caused by individual age and experiences. The video observation of wild boar revealed important behavioural differences - presumably causing bias in the individual sampling probabilities - which otherwise would not have been detected. Thus, video observation allowed evaluating the feasibility of hair sampling via hair traps for this species without need to analyze hair samples in the laboratory. To our knowledge, in none of the studies mentioned above video observation was used to evaluate the behaviour of the animals visiting the sampling stations. Thus, potential heterogeneities in the individual sampling probabilities might remain undetected.

In our pilot study, we applied a sampling strategy which presupposes that the animals actively approach a baited sampling station. In social species, this might provoke behavioural interactions between individuals visiting a station and thus result in differences in the sampling probability caused by age, social status and individual experiences. These findings suggest that such “active” sampling strategies may be less suitable for use in population estimation of social species, causing increased heterogeneity bias. In contrast to this, “passive” sampling strategies in which the tissue sample is obtained where the animals left or deposed it without any behavioural manipulation (e.g. collection of faeces along transects) might allow a more representative survey especially of social species. “Active” sampling at baited stations may even cause behavioural responses comparable to those occurring in classical capture-mark-recapture (‘trap happy’ or ‘trap shy’ individuals, see e.g. Boulanger et al. 2004; and C. Ebert, personal observation).

Irrespective of the heterogeneity which is present in the hair sampling behaviour of wild boar, the efficiency and practicability of hair sampling via baited sampling stations also depends on the sampling grid density and thus on the effort necessary to obtain a sufficiently high sampling probability. Settlage et al. (2008) showed that hair sampling is not suitable to yield an accurate population estimate for black bears

due to their small home ranges compared to grizzly bears. To account for those small home ranges, the sampling effort has to be considerably higher. The situation seems similar concerning wild boar: GPS based telemetry carried out in our study area between September 2006 and January 2008 on 6 adult wild boar indicate mean 1-month home range sizes of 474 ha for males and 192 ha for females (95 % MCP; Ebert, unpublished data). These data suggest a minimum sampling density of 1 station per 200 ha, which will be difficult to realise on a larger scale. Radiotracking data obtained from wild boar in other regions of Europe and the USA support this result (reviewed in Keuling et al. 2007).

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Chapter 3

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[3]

Abstract

The 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.

Keywords: mark-recapture, genotyping, transect, sample size, population density

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

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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. 2010): 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).

Material and methods

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.

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).

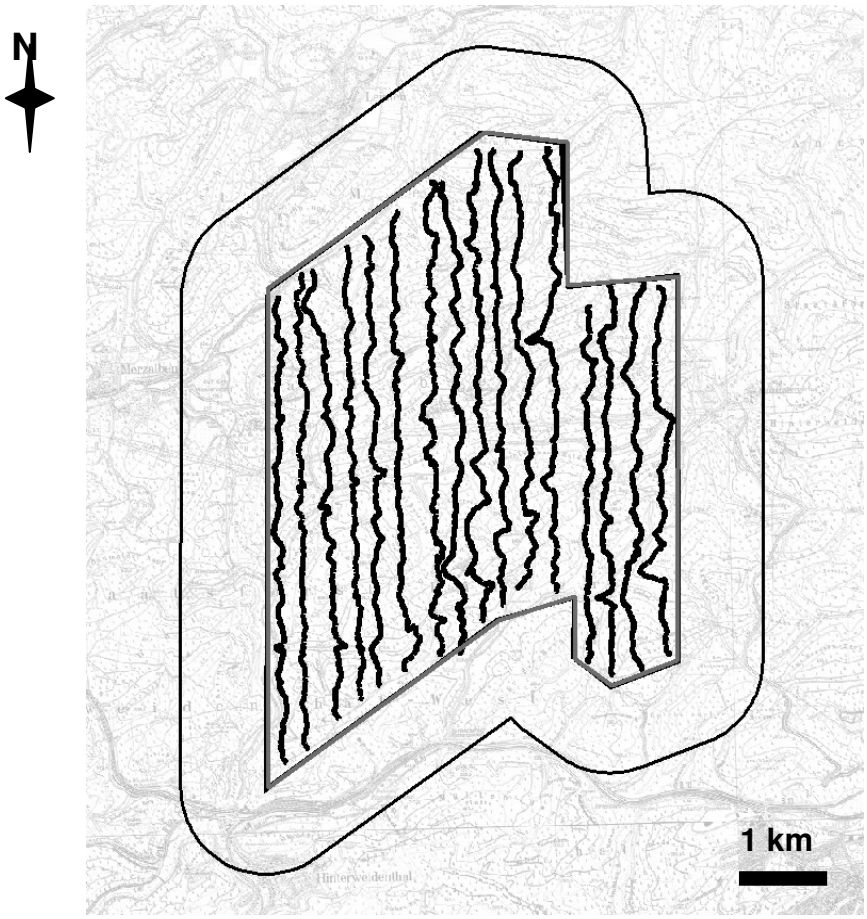

Figure 1: Transect design in the study area (25 km²) and buffer with the width of a mean monthly wild boar home range radius marking the effective sampling area (52 km²)

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.

RESULTS

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).

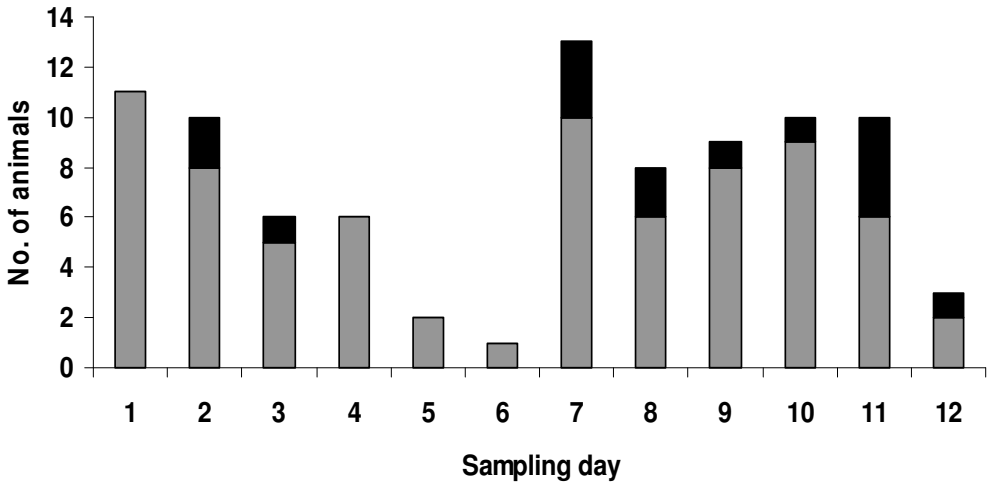

Figure 2: Number of wild boar sampled per day. The number of animals sampled first time is given in grey, recaptures are given in black.

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

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).

Discussion

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).

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.

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.

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 sample size will have to be increased considerably. Furthermore, 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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Chapter 4

Estimating wild boar (*Sus scrofa* L.) population size using faecal DNA and capture-recapture modelling [4]

Abstract

Increasing populations of wild boar and feral domestic pigs *Sus scrofa* L. have evoked growing concern due to their potential as disease reservoir and as an origin of agricultural damages. Reliable population estimates are needed to calibrate hunting regimes or other management measures for this species. As an alternative to traditional methods, non-invasive genetic population estimation approaches based on hair or faeces sampling have yielded promising results for several species in terms of feasibility and precision. We developed and tested a non-invasive population estimation approach based on wild boar faeces in a study area situated in the Palatinate Forest, south western Germany. Faeces were collected along transects during December 2006 and December 2007 in two separate trials. The number of samples collected per km was 0.23 and 0.53 respectively. Genotyping was carried out using five microsatellite markers to discriminate between individuals. During the 2006 and 2007 trials, 75 and 132 individual wild boar were identified, respectively. In both data sets, capture probabilities and recapture rates were low. Using multimodel inference and model averaging, we obtained relatively consistent estimates for the 2007 data set, whereas the results differed considerably between the applied models for the 2006 data set. As a basis for management decisions, we focused on the most conservative estimates in order to avoid overestimating the population. Population densities calculated using estimates derived from the most conservative model were 4.1 wild boar per km² (2.8 – 5.9) for 2006 and 9.1 (5.6 – 11.4) for 2007. In the future, to further improve reliability and precision of population estimates based on wild boar faeces, the sample size will have to be increased. However, even when considering the most conservative of the population estimates, results show that the present hunting regime in our study area is not effective in regulating the wild boar population. The method presented here offers a tool to calibrate hunting or other management measures for wild boar.

[4] Corresponding publication: Ebert, C., Kolodziej, K., Schulz, H.K., Hohmann, U.: Estimating wild boar (*Sus scrofa* L.) population size using faecal DNA and capture-recapture modelling. Submitted to *Wildlife Biology*.

Keywords: genotyping; individual identification; population density; transects, sample size; wildlife management

Introduction

Wild boar and feral domestic pigs (both *Sus scrofa* L.) have moved into the focus of wildlife management in many countries worldwide (Bieber & Ruf 2005). Population numbers are rapidly increasing, resulting in agricultural and other damages and also in increased spread of diseases like e.g. the Classical Swine Fever or Aujeszki's disease (Acevedo et al. 2007; Saez-Royuela & Telleria 1986; Schley et al. 2008; Toigo et al. 2008). At present, the main regulatory mechanism for the growing wild boar populations is hunting, especially in regions where natural predators are lacking like in most parts of Central Europe (Boitani et al. 1995b; Toigo et al. 2008). However, until now, there is no efficient method to assess the effectiveness of a given hunting regime in regulating wild boar populations. Hunting bags do not necessarily reflect actual population sizes. Furthermore, it is crucial to obtain reliable and absolute population estimates in order to enable efficient management measures and for epidemiologic reasons (Baber & Coblentz 1986; Sweitzer et al. 2000; Truvé 2004; Acevedo et al. 2007). Based on reliable population estimates, modified hunting regimes or other regulatory mechanisms, such as fertility control via immunocontraceptives, could be enforced (Massei et al. 2008). Hunting bag statistics and other traditional approaches such as counts of tracks, faeces or farrowing nests yield only relative numbers or population trends (Acevedo et al. 2007). Capture-mark-recapture (CMR) approaches can yield absolute population numbers (Otis et al. 1978; Seber 1982, Pollock et al. 1990). However, relatively few CMR studies have been carried out on wild boar (but see Andrzejewski & Jezierski 1978; Baber & Coblentz 1986). Reasons might be that CMR is barely feasible for a large, difficult to capture and elusive species like the wild boar and furthermore bears a high risk of yielding severely biased results, because capture probabilities are influenced by age, sex and social status (Petit & Valière 2006; Briedermann 2008). In this context, non-invasive DNA-based methods, which have been widely applied for estimation of population size (often using a CMR framework) in the last years, have yielded promising results (Taberlet et al. 1999; Beja-Pereira et al. 2009). They are said to be particularly advantageous in case of rare or endangered animal species, because for those obtaining reliable population estimates with conventional methods is especially difficult,

and in some cases the risk of damage through invasive approaches like e.g. removal methods or CMR may prohibit their use (Puechmaille & Petit 2007; Jacob et al. 2009). However, non-invasive genetic methods may also be beneficial for population estimation of abundant species like the wild boar, because they may yield less biased and more representative estimates compared to most traditional approaches (McKelvey & Schwartz 2004; Fickel & Hohmann 2006; Petit & Valière 2006; Zhan et al. 2006). Nevertheless, several issues are crucial for the successful application of non-invasive methods. One is to ensure a reliable laboratory protocol with careful error-checking for DNA sample analysis, because genotyping errors like allelic dropout and false alleles can severely compromise population estimation (Creel et al. 2003; Lukacs & Burnham 2005b). Furthermore, it may be difficult to obtain a sufficiently large sample size and a sufficiently high detection probability with a feasible effort (Ebert et al. 2009; Harris et al. 2010).

For mammals, the main sources for non-invasively obtainable DNA samples are hairs and faeces. After having conducted pilot studies of both hair and faeces sampling for wild boar (Ebert et al. 2009 and 2010), we decided to focus on faeces in case of this species, because hair sampling using baited hair traps seemed to be strongly influenced by individual age and group status of the animals.

In this paper, the results of two faeces sampling trials are shown with the aim to evaluate the suitability of non-invasive genetic population estimation via faeces sampling for wild boar management. In particular, the estimated population numbers are used to evaluate the hunting regime in the study area with respect to its success in regulating wild boar numbers.

Material and methods

STUDY AREA

Faeces sampling was carried out in a site of 2500 ha situated in the Palatinate Forest in the federal state of Rhineland-Palatinate, south western Germany (49°12'N, 7°45' E). Elevation ranges between 210 m and of 609 m. 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 in 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. The annual harvest of wild boar in the state-hunting areas between 1999 and 2009 averages 2.4 individuals per km² (Range: 1.14 to 5.23 individuals per km² and year; Reis 2006; G. Scheffler, Forestry of Hinterweidenthal, personal communication). The hunting bag in the first study year 2006 was comparably low (1.8 wild boar per km²). Contrastingly, in 2007 the hunting bag was relatively high (3.9 wild boar/ km²). Hunting is carried out both via single hunt all year round and via drive hunts. The latter are carried out in segments of three to five km² between mid-October and the end of January every two to three weeks, covering the whole study area during one season. The mean frequency of raised stand occupancy for the single hunt is 47 per km² and year. In drive hunts, the average number of hunters and dogs per km² is 17 and 5.6 respectively.

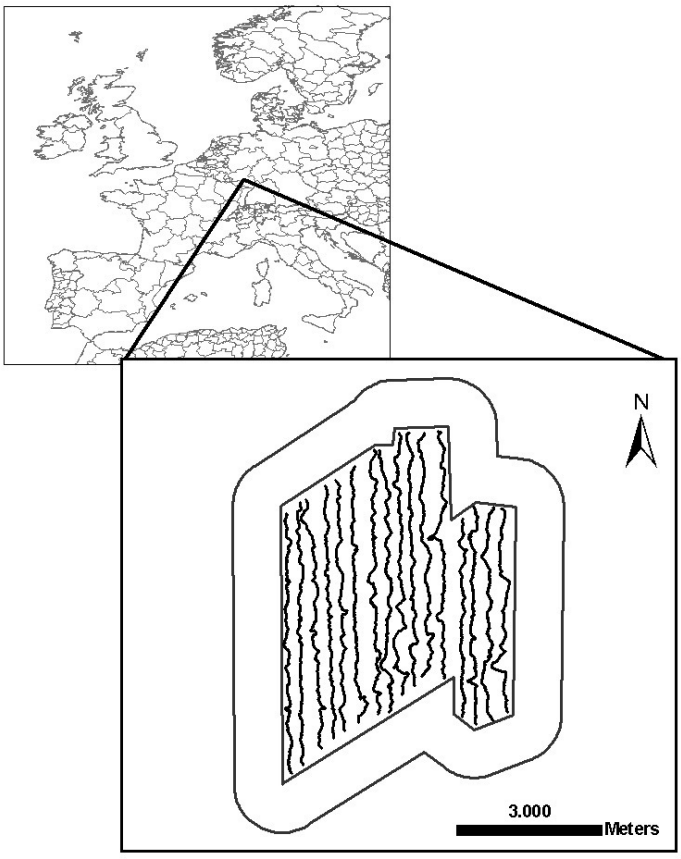

Figure 1: Transect design for collection of wild boar faeces for use in non-invasive genetic population estimation. The transects are orientated in N-S direction. The area covered by transects together with the buffer represents the effectively sampled area.
The study area is situated in the federal state of Rhineland-Palatinate in south western Germany

FIELD SAMPLING

Sampling was carried out between November 27th and December 12th 2006 and November 27th and December 13th 2007. Wild boar faeces were collected along 16 transects of approx. 6 to 8 km length each (Fig. 1). Transects were installed parallel to each other in north-south orientation (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. We aimed to maximize the collection of fresh faeces by walking the same transect routes in every repetition. Transect routes 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. Each transect was searched for faeces every 48 hours and thus a total of six times during 12 days in one trial.

For the 2007 sampling trial, we modified the sampling method with the purpose to increase the faeces finding rate by applying a simplified form of adaptive cluster sampling (Thompson 1991). Every time a faeces sample was found, the field worker paused walking the transect and searched the area surrounding the sample in a radius of approximately five to six meters. If further wild boar faeces were found within this radius, it was extended in the respective direction. After having completed a cluster, the field worker continued walking the regular transect route. Thereby, we aimed to account for the fact that wild boar – at least females and their offspring – regularly occur in groups and thus often faeces of more than one individual can be found in close proximity (Briedermann 2008).

Whole faeces were collected using inverted freezer bags, which were then reversed and closed. Samples were stored frozen (-19ºC) in the sealed freezer bags until analysis.

DNA EXTRACTION AND GENOTYPING

Genomic DNA was extracted from all faecal samples using the NucleoSpin tissue kit (Macherey-Nagel, Düren, Germany) modified in that the wash step during DNA

purification was tripled. Individual identification was carried out based on 4 microsatellite loci: TNFB (Lowden et al. 2002), SW2496, SW2021 and SW742 (Rohrer et al. 1994). For sex determination, a Y-linked sex marker was used (PigSRY, Kawarasaki et al. 1995). We calculated the probability of identity (P_{ID}) and the P_{ID} between siblings (P_{IDsibs}) using GIMLET (Valière 2002) to test if the combination of loci is sufficient to discriminate between individuals for the purpose of population estimation in our studied population (Woods et al. 1999; Frantz et al. 2003). A comparative multitube approach was applied for genotyping (Taberlet et al. 1999), with up to 10 replicates for each sample. A sample had to yield at least eight identical replicates to be considered as homozygous at a given locus and three identical replicates to confirm a heterozygous locus. All samples which failed to amplify for one ore more loci were discarded from further analysis. Genotyping results were analysed using GENECAP (Wilberg & Dreher 2004).

Genotyping error rate was determined by conducting a blind test. For this, we collected a number of fresh wild boar faeces within one day in the field at locations with more than 12 km linear distance from each other in order to ensure samples originated from different individuals. We then partitioned each faeces sample into three to six subsamples, resulting in a total of 40 subsamples. These were randomly numbered and sent to the laboratory for genotyping. The subsamples were then assigned – according to their genotypes – to the different individuals by the lab personnel who had no information about the real origin of each subsample. Of the 23 subsamples which yielded usable genotypes, 22 were assigned correctly and one was assigned erroneously to a spurious individual due to allelic dropout, resulting in a per sample-error rate of 4.3%. No false alleles were observed in the blind test, thus we assume the false allele rate to be sufficiently low for the purpose of population estimation. The blind test furthermore resulted in changing the criteria for determining the sex of genotypes, because the erroneous genotype, which belonged to the sample of a male, was wrongly identified as a female due to dropout of the Y-linked sex marker (according to the original protocol the result ‘male’ had to be confirmed in two PCR replicates). After the blind test, up to six additional replicates were carried out for the PigSRY marker in samples which showed the Y-band only once or showed no band after the first two replicates. The blind test had been carried out after the 2006 samples had been analysed and presented in a pilot study (cf. Ebert et

al. 2009), but before the analysis of the 2007 samples. Therefore, additional replicates of the sex marker were carried out for all 2006 samples. Due to this modification in the sex determination protocol, the sex ratio in the 2006 data has changed from 1 : 1.84 when they had been first presented (Ebert et al. 2009) to 1.14 : 1 in this study. Obviously, the number of males had been underestimated using the original protocol.

POPULATION SIZE ESTIMATION

We used closed capture models in program MARK (White & Burnham 1999) to estimate population size. We defined a set of plausible candidate models with varying assumptions concerning capture probability (p), chosing nomenclature according to Otis et al. (1978) for simplicity: M Null as the most parsimonious model with capture probablity being constant over time and among individuals, M h (heterogeneity; a mixture model incorporating two groups of animals with differing p), M t (p varying over time) and M th (heterogeneity and p varying over time). For each of these basic models, we considered 4 different cases:

- basic model
- basic model including sex (two attribute groups)
- basic model including 5% misidentification due to genotyping error (Lukacs & Burnham 2005b); we chose 5% instead of the 4.3% error rate determined via blind test in order to be more conservative)
- basic model including sex and 5% misidentification

Thus, for each data set (2006 and 2007), we calculated estimates using 16 different models. Furthermore, for each of the two data sets, we constructed capture histories for population estimation in two ways: For the first, we included only detections of the same individuals on different sampling days as ’recaptures’, i.e. multiple captures during one sampling day were pooled to a single capture for each of the 12 sampling days. In the following, we will refer to this approach as ’detections on different days only’, or DDO. For the second, we included all possible recaptures, i.e. plus multiple detections of individuals on the same day (see also Miller et al. 2005, Ruell et al. 2009) and shifted one of these detections back in time one day to fit in the capture history (the maximum number of detections recorded for the same individual per day

was two). We only included detections of the same individual and the same day that were spatially separated by at least 250 m from each other in order to avoid pseusoreplicates (Miller et al. 2005). We will refer to the second approach as 'maximum number of detections', or MND.

For every data set and each of the two approaches, we calculated population size using all 16 models. Additionally, we calculated model averages (i.e. weighted average over all models according to their model weight and thus according to their GOF; Burnham & Anderson 2002). Since in program MARK, confidence intervals (CI) for model averages do not account for the minimum number of wild boar observed in the sampling area, we calculated CI using the unconditional SE and the equations reported in Rexstad & Burnham (1992; page 19). For management reasons, we additionally aimed to obtain CI for the total population (male + female). Program MARK estimates population sizes and CI separately for both sexes, when sex is included as a grouping variable as it is the case in our analysis. Therefore, we calculated the sum of a random number of the female and male probability distribution, iterated this 10.000 times and calculated mean (total population size) and standard error from the resulting distribution. We used mean and standard error to calculate 95%-CI's based on the corresponding Rexstadt & Burnham (1992) equations.

POPULATION DENSITY

For purpose of comparison with other wild boar populations and other studies as well as for comparison with the hunting bag per km² in the study area, we calculated population densities. Due to the short time span of sampling and because there were no drive hunts and most probably no births during each sampling trial, we consider the assumption of demographic closure as met in our study (Otis et al. 1978). However, there was no possibility to obtain topographic boundaries for the study area, thus it can not be considered as geographically closed. The small sample size prevented us from applying models for open populations for population estimation (Luikart et al. 2010). Therefore, we added a buffer zone around the transect grid to calculate the effectively sampled area (ESA). The ESA was then used for calculating population density. The width of the buffer was determined using VHF- and GPS-telemetry data collected from wild boar tracked in our study area (C. Ebert,

unpublished data). We chose the radius of a mean monthly home range (95% Minimum Convex Polygon) as a buffer for calculating the ESA (see e.g. Tioli et al. 2009). This resulted – with a mean monthly home range radius of 1000 m – in an ESA of 52 km².

To evaluate the efficiency of the hunting regime in our study area, we calculated an estimate of the reproductive output for both study years for comparison to the hunting bags for the given year. To calculate reproductive output, we assumed a population growth rate of 200% per year which was derived from combined data on wild boar reproduction in our study area and in a similar forested habitat also situated in southwestern Germany (Gethöffer, Sodeikat & Pohlmeyer 2007). As a basis for the calculation of reproductive output, we used the lower confidence interval of the MNO model M t 5% mis for each study year. We used this very conservative approach in order to avoid overestimating population size and thus population output and therefore to compare a minimum output to the hunting bag. Overestimation of population size is one of the pitfalls of non-invasive genetic approaches when genotyping error is present (Creel et al. 2003), furthermore the danger of obtaining biased estimates is increased when data are sparse like in our study (Pollock 1990).

Table 1: Overview over results of faeces sampling in a wild boar population in the Palatinate Forest, SW Germany

		Male	Female	Both sexes
Sampling 2006	No. of samples genotyped successfully	50	39	89
	No. of individuals	40	35	75
	No. recaptures on different days only	10	2	12
	No. all possible recaptures	11	2	13
Sampling 2007	No. of samples genotyped successfully	80	76	156
	No. of individuals	67	65	132
	No. recaptures on different days only	7	9	16
	No. all possible recaptures	8	11	19

Results

FIELD SAMPLING AND GENOTYPING

During the 2006 sampling period, we collected 141 wild boar faeces (i.e. 0.23 samples per km of transect searched). Of these, 89 (63 %) yielded a complete 5-

locus consensus genotype. From these, we identified 75 individual animals, 40 males and 35 females. In 2007, 326 faecal samples were collected (i.e. 0.53 samples per km of transect searched). Of these, 156 (47.8 %) were genotyped successfully, representing 132 individual wild boar of which 67 were males and 65 were females (Table 1). P_{ID} and P_{IDsibs} were 2.93 x 10^{-5} and 0.0156, respectively, thus the set of markers allows discrimination between individuals with sufficient certainty for our purpose (Lukacs & Burnham 2005b, Woods et al. 1999). In 2006, 11% (N = 8) of all individuals and in 2007 10% (N = 13) of all individuals were detected more than once, respectively. The number of detections per individual ranged from 1 to 5 in both years. The sex ratio of the sample was nearly balanced with slightly more males in both years (1.14 : 1 male to female in 2006 and 1.03 : 1 in 2007 respectively). In 2006, by far the most individuals which were detected more than once (i.e. 'recaptured') were males (table 1). This was not the case in 2007.

Table 2: Top five candidate models for population estimates of wild boar in the Palatinate Forest, derived from faeces sampling in December 2006 and calculated using program MARK. Model selection is based on Akaike's Information Criterion corrected for small sample sizes (AICc). Further parameters given are w_i (model weights), K (number of parameters), and N (estimated population size) for both sexes including 95 % confidence intervals. For a detailed description of the estimation models see text. DDO = capture history created using detections on different days only; MND = capture history created using maximum number of detections

Data set	Model	K	Δ AICc	w_i	$\hat{N}$ male	$\hat{N}$ female	$\hat{N}$ total	Both sexes SE	Both sexes 95% CI	Density
DDO	M h 5%	5	0	0.431	414	363	777	621	225-3182	14.9
	M h	5	0.055	0.419	470	411	881	719	247-3678	16.9
	M h sex 5%	8	4.490	0.045	1467	264	1731	11740	101 - 80979	33.3
	M h sex	8	4.494	0.045	1776	294	2070	15376	105 - 105149	39.8
	M t 5%	15	6.607	0.016	122	107	229	44	156 - 334	4.4
	Model average				531	363	894	4174	90 - 28800	15.0
MNO	M h 5%	5	0	0.420	285	249	534	307	179 - 1204	10.3
	M h	5	0.046	0.410	321	281	602	349	236 - 1793	11.6
	M t 5%	15	4.337	0.048	115	100	215	39	156 - 314	4.1
	M h sex	8	4.338	0.045	370	294	664	694	169 - 3742	12.8
	M t	15	4.820	0.036	126	110	235	44	169 - 346	4.5
	Model average				277	247	526	316	187 - 1757	10.1

POPULATION SIZE ESTIMATION

Sampling 2006

For the 2006 sampling data, the most supported model included heterogeneity and a misidentification rate of 5%. The model averaged population estimate in case of DDO indicates a total population size of 894 wild boar (Table 2; for results of all 16 models for both approaches see Appendix S1 in supporting information). The two most supported models show very large confidence intervals and standard errors compared to the Mt and M 0 models, which furthermore yield considerably lower population estimates. In case of MND, the model averaged population estimate indicates a total population size of 526 wild boar (Table 2). Confidence intervals and standard errors for the two most supported models are still very large, albeit considerably smaller compared to the DDO approach.

Table 3: Top five candidate models for population estimates of wild boar in the Palatinate Forest, derived from faeces sampling in December 2007 and calculated using program MARK. Model selection is based on Akaike's Information Criterion corrected for small sample sizes (AICc). Further parameters given are w_i (model weights), K (number of parameters), and N (estimated population size) for both sexes including 95 % confidence intervals. For a detailed description of the estimation models see text. DDO = capture history created using detections on different days only; MND = capture history created using maximum number of detections

Data set	Model	K	Δ AICc	w_i	$\hat{N}$ male	$\hat{N}$ female	$\hat{N}$ total	Both sexes SE	Both sexes 95% CI	Density
DDO	M t 5%	16	0	0.544	238	236	474	76	354 - 657	9.1
	M t	16	0.489	0.426	262	260	522	85	387 - 727	10.0
	M th 5%	27	7.242	0.015	296	287	583	113	410 - 863	11.2
	M th	27	7.539	0.013	326	316	642	127	447 - 956	12.4
	M t sex 5%	28	12.308	0.001	258	217	475	104	324 - 745	9.1
	Model average				250	248	498	85	366 - 705	9.6
MNO	M t 5%	16	0	0.353	211	204	415	61	318 - 561	7.9
	M th	27	0.633	0.258	275	267	542	92	398 - 765	10.4
	M t	16	0.803	0.237	232	225	457	67	350 - 616	8.8
	M th 5%	27	1.758	0.147	296	287	583	110	413 - 854	11.2
	M t sex 5%	28	9.396	0.032	234	185	419	83	297 - 632	8.1
	Model average				245	238	483	93	343 - 716	9.3

Sampling 2007

For the 2007 sampling data, the most supported model includes variation in p over time as well as 5% misidentification. The total model averaged population size for DDO is 498 wild boar. For the MND approach, the model average yielded a total population size of 483 wild boar (Table 3; for results of all 16 models for both approaches see Appendix S2 in supporting information).

POPULATION DENSITY

Sampling 2006

Population density calculated using the model averaged population estimates is 15.0 wild boar per km² (95% CI 6.4 – 66.1) for the DDO approach and 9.9 wild boar per km² (95% CI 2.8 – 31.6) for the MNO approach (Table 2). When considering only model M t 5% mis (because it is the model with the lowest deviation, i.e. best fit to the data, and for purpose of comparison with the 2007 data), the estimated population densities for 2006 are quite similar for the two approaches: 4.4 (95% CI 3.0 – 6.4) for DDO and 4.1 (95% CI 2.8 – 5.9) for MNO.

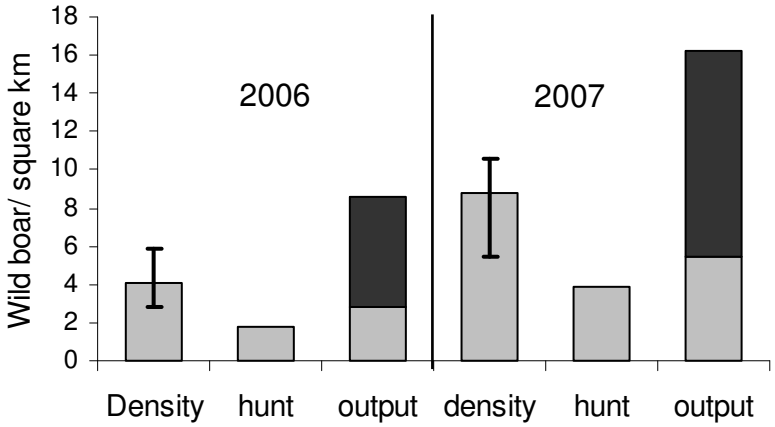

Figure 2: Comparison between estimated wild boar population density (MNO approach, Model M t 5% misidentification; error bars indicate 95% CI), hunting bag and estimated population growth (reproductive output) in the corresponding study area and study year. Reproductive output (dark grey) was calculated based on the lower CI of estimated population density

Sampling 2007

For the 2007 data set, the estimated density for the DDO approach is 9.6 wild boar per km² (95% CI 5.8 – 12.3; Table 3). For the MNO approach, the estimated density is 9.9 wild boar per km² (95% CI 5.8 – 13.4). When considering only M t 5% mis as the most supported model, the densities are 9.1 (95% CI 5.6 – 11.4) for DDO and 9.3 (95% CI 5.3 – 12.5) for MNO, respectively.

Discussion

For both data sets the capture probabilities (p) were very low (mean p ranged between 0.022 and 0.030 depending on the approach). Thus the population coverage (i.e. the proportion of the population represented in the sample) and the number of recaptures were also low. Since capture probabilities have to be sufficiently high (minimum $p > 0.1$, better $p > 0.2$) in order to yield reliable and precise estimates (Otis et al. 1978), the model selection and the population estimates presented in this paper may be of limited power. For the 2006 data set, this is reflected in the large differences between the estimates generated using different models. In contrast to this, the 2007 data set yielded more consistent results, differences in estimated population size being considerably smaller between models and also between DDO and MNO. For both data sets, the estimates generated using the M h and M h sex models yield implausibly large population numbers and CI (see Appendix S1 and S2).

Wild boar have a low defecation rate compared to other ungulates (Briedermann 2008). In a study carried out in the same area, three to four times as many red deer faeces were collected with a similar effort compared to the wild boar sampling trials, even though red deer density is considerably lower (C. Ebert, unpublished data). Furthermore, the climate in our study area is rather mild and humid, which limits sample persistance in the field and DNA quality and thus limits sample size (Luccini et al. 2002, Murphy et al. 2007). In further studies, sample size will have to be increased, e.g. by increasing sampling intensity or by using other approaches in addition to faeces sampling. The latter may not only be valuable to increase the sample size, but also to yield data with low overall sampling bias (Dreher et al. 2007, Boulanger et al. 2008). For wild boar, this could be achieved by collecting tissue samples from hunted individuals or by obtaining hair samples using hair traps or rub

trees. Hair sampling at hair traps may not be suitable as a sole strategy for wild boar population estimation (as indicated in our pilot study; Ebert et al. 2010), but serve as an additional sample. It may also be promising to stratify the faeces sampling by searching more intensively along wild boar passes, at wallows or feeding sites in addition to walking transects. Such incidental or opportunistic sampling can provide a valuable additional sample (Gervasi et al. 2008). Since in our study we had no possibility to increase the sample size using additional strategies, we applied the MNO approach in order to exploit all the available capture information. We consider the MNO approach in the case of very sparse data as useful and assume that in our case it has improved the estimates, because the MNO estimates have smaller standard errors and narrower confidence intervals compared to those generated using the DDO approach (tables 2 and 3).

Instead of using the MNO approach, we could have calculated population estimates using program CAPWIRE (Miller et al. 2005). Like the MNO approach and in contrast to the traditional CMR (DDO) approach, CAPWIRE allows using every single observation of each individual for population estimation. Furthermore, CAPWIRE is assumed to be robust to time variation in p and is said to perform well in presence of individual heterogeneity. However, CAPWIRE is well suited for populations below 100 individuals, but tends to produce overestimates for large populations and when sample size is low (Miller et al. 2005). In our case, the sampled populations most probably were too large and the recapture rates too low for CAPWIRE to function properly. Furthermore, the presence of genotyping errors can additionally inflate CAPWIRE estimates.

To some degree, incertainty due to genotyping errors is most probably present in our data. We believe the overall misidentification rate to be appropriately reflected by the result of the blind test and thus not larger than 4 to 5% due to the careful genotyping approach with up to ten PCR replicates per locus. However, in simulation tests carried out by Roon, Waits & Kendall (2005b), population estimates derived using heterogeneity models were heavily inflated in presence of genotyping errors, even for low error rates. This upward bias was considerably higher for heterogeneity models compared to e.g. the M Null estimator. In our study, the estimates based on M h models suggest much larger populations than those derived from M t or M Null

models. According to the findings of Roon, Waits & Kendall (2005b), the M h and especially M h sex population estimates might considerably overestimate the actual population size in our study area. To account for the presence of genotyping error in our data, we included models incorporating misidentification through genotyping error for each of the four applied model types. In the model selection, these generally outranked the corresponding models that did not include misidentification. However, the difference in GOF and in the estimated population size between models of the same type with and without misidentification (e.g. M t and M t 5% mis) was small (ΔAICc <1; difference in estimated N between 7% and 13%, mean 10%). This compensation might not be sufficient to account for bias in M h models, which can be considerably higher (Creel et al. 2003; Roon, Waits & Kendall 2005). Therefore, we consider the estimates generated using models M Null or M t as more reliable and less biased than those derived from heterogeneity models, facing the presence of genotyping error in our data. Heterogeneity models were not supported in the 2007 data set, but ranked high in the model selection for the 2006 data. One main reason for this finding is probably the strong imbalance between the sexes concerning recaptures in the 2006 data set. However, this may be an artefact of the small sample size, as it is not the case in 2007, where there was no incidence for heterogeneity. Thus, we do not believe the model selection for 2006 to be valid. For very sparse data like our 2006 data set, simple and parsimonious models often yield less biased results compared to the more elaborate models, even if there is no misidentification (Menkens & Anderson 1988; McKelvey & Pearson 2001). The estimates based on Mt 5% mis, M Null and Mt correspond much more to the relation of sample size, hunting bag and population size between the two study years. Nevertheless, we have no possibility to test which of the models reflects reality best for the given data. However, since in our case it seems more probable that the M h models represent an overestimation of population size, we consider it reasonable to base management implications on conservative estimates and thus focus on the M t 5% mis model for both the 2006 and 2007 data set when considering management measures for the area.

MANAGEMENT IMPLICATIONS

We selected a minimum estimate of population density as well as a moderate reproduction rate (Bieber & Ruf 2005) to calculate reproductive output for comparison

to the hunting bag in the study area. Even when using this very conservative approach, the number of harvested wild boar corresponded only to approximately 35% of the estimated reproductive output (Fig. 2). Thus, the current hunting regime in our study area does not seem to be efficient in regulating the wild boar population, even though the forestry in charge aims at reducing the population (G. Scheffler, forestry of Hinterweidenthal, personal communication). Since 1999, the mean hunting bag in the study area has increased almost threefold. To anticipate a further increase in population size and even more to reduce the wild boar numbers either the hunting regime will have to be changed (e.g. hunt more females of all age classes; Toigo et al. 2008) or other regulatory mechanisms will have to be established (e.g. contraceptives, Massei et al. 2008).

Our study area is only one example – it is a known problem in many regions that hunting is not efficient in regulating wild boar populations. Nevertheless, until now there has been no measure for the extent to which hunting can achieve a reduction of a population or how far it is away from achieving a sufficient reduction. The method presented in this paper represents a tool to quantify the success of hunting or other management measures and thus serve as a calibration for wild boar management.
However, in order to allow more reliable and precise population estimates and thus more fine-grained conclusions for management, the sample size will have to be increased. Further studies should focus on the development of sampling strategies that allow a better representation of the sampled population in terms of number of unambiguously identified genotypes. Thus, faeces sampling efficiency and the combination with other strategies (e.g. genetic sampling of the hunting bag) are relevant parameters for research, but also the improvement of genotyping success and further reduction of the genotyping error rate.

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Appendix S1: Overview over all candidate models for population estimates of wild boar in the Palatinate Forest, derived from faeces sampling in December 2006 and calculated using program MARK. Model selection is based on Akaike's Information Criterion corrected for small sample sizes (AICc). Further parameters given are w_i (model weights), K (number of parameters), and N (estimated population size) for both sexes including 95 % confidence intervals. For a detailed description of the estimation models see text. DDO = capture history created using detections on different days only; MND = capture history created using maximum number of detections

Data set	Model	K	Δ AICc	w_i	$\hat{N}$ total	SE	95% CI
DDO	M h 5%	5	0	0.431	777	621	233 - 3190
	M h	5	0.055	0.419	881	719	255 - 3686
	M h sex 5%	8	4.490	0.045	229	11740	109 - 80979
	M h sex	8	4.494	0.045	2070	15376	113 - 105149
	M t 5%	15	6.607	0.016	229	44	164 - 342
	M t	15	7.135	0.012	253	48	181 - 374
	M th 5%	27	7.762	0.010	313	74	206 - 507
	M th	27	7.955	0.008	345	82	226 - 558
	M 0 sex 5%	6	9.275	0.004	353	178	163 - 952
	M 0 sex	6	9.737	0.003	392	198	178 - 1051
	M 0 5%	4	9.792	0.003	233	45	166 - 348
	M 0	4	10.339	0.002	257	50	182 - 384
	M t sex 5%	28	13.636	0.001	343	173	159 - 927
	M t sex	28	14.113	0.000	379	194	171 - 1030
	M th sex	52	46.386	0.000	419	199	195 - 1060
	M th sex 5%	52	48.612	0.000	418	187	201 - 999
	Model average				793	667	227 - 3438
MNO	M h 5%	5	0	0.420	534	307	179 - 1204
	M h	5	0.046	0.410	602	349	236 - 1793
	M t 5%	15	4.337	0.048	215	39	156 - 314
	M h sex	8	4.338	0.045	664	694	169 - 3742
	M t	15	4.820	0.036	235	44	169 - 346
	M th 5%	27	6.094	0.020	283	62	196 - 1766
	M th	27	6.244	0.019	312	121	267 - 684
	M 0sex 5%	6	6.745	0.014	347	180	158 - 960
	M 0 sex	6	7.138	0.012	385	200	173 - 1060
	M 0 5%	4	8.097	0.007	219	40	159 - 321
	M 0	4	8.612	0.006	242	44	176 - 352
	M t sex 5%	28	10.506	0.002	338	175	155 - 937
	M t sex	28	10.900	0.002	373	191	169 - 1016
	M th sex	52	43.837	0.000	404	196	187 - 1044
	M th sex 5%	52	47.987	0.000	343	174	159 - 932
	Model average				526	316	195 - 1765

Appendix S2: Overview over all candidate models for population estimates of wild boar in the Palatinate Forest, derived from faeces sampling in December 2007 and calculated using program MARK. Model selection is based on Akaike's Information Criterion corrected for small sample sizes (AICc). Further parameters given are w_i (model weights), K (number of parameters), and N (estimated population size) for both sexes including 95 % confidence intervals. For a detailed description of the estimation models see text. DDO = capture history created using detections on different days only; MND = capture history created using maximum number of detections

Data set	Model	K	Δ AICc	w_i	$\hat{N}$ total	SE	95% CI
DDO	M t 5%	16	0	0.544	474	76	354 - 657
	M t	16	0.489	0.426	522	85	387 - 727
	M th 5%	27	7.242	0.015	583	113	410 - 863
	M th	27	7.539	0.013	642	127	447 - 956
	M t sex 5%	28	12.308	0.001	475	104	324 - 745
	M t sex	28	12.797	0.009	522	116	352 - 822
	M th sex 5%	52	34.879	0.000	694	223	398 - 1321
	M th sex	52	44.944	0.000	654	188	395 - 1167
	M h 5%	5	47.400	0.000	4153	18410	260 - 125913
	M h	5	47.559	0.000	5146	25790	276 - 177590
	M h sex 5%	8	52.313	0.000	28426	504155	389 - 3129161
	M 0 5%	4	53.746	0.000	495	81	368 - 691
	M 0	4	54.356	0.000	548	91	404 - 767
	M 0 sex 5%	6	57.682	0.000	498	110	338 - 783
	M 0 sex	6	58.292	0.000	550	121	372 - 860
	M h sex	8	60.388	0.000	35634	250996	798 - 1730160
	Model average				498	85	366 - 705
MNO	M t 5%	16	0	0.353	415	61	318 - 561
	M th	27	0.633	0.258	542	92	398 - 765
	M t	16	0.803	0.237	457	67	350 - 616
	M th 5%	27	1.758	0.147	583	110	413 - 854
	M t sex 5%	28	9.396	0.032	419	83	297 - 632
	M t sex	28	10.195	0.002	461	94	257 - 637
	M th sex 5%	52	19.128	0.000	609	172	376 - 1074
	M th sex	52	34.592	0.000	619	184	370 - 1128
	M h 5%	5	34.593	0.000	1630	1295	413 - 6544
	M h	5	34.789	0.000	1842	1475	414 - 6529
	M h sex 5%	8	38.489	0.000	10130	91412	296 - 621092
	M h sex	8	47.257	0.000	984	629	366 - 3171
	M 0 5%	4	51.392	0.000	433	65	330 - 589
	M 0	4	52.351	0.000	479	71	365 - 648
	M 0 sex 5%	6	55.158	0.000	438	88	308 - 664
	M 0 sex	6	56.119	0.000	484	98	338- 733
	Model average				483	93	343 - 716

Chapter 5

Estimating red deer (*Cervus elaphus*) population size based on non-invasive genetic sampling[5]

Abstract

1. Some deer species are of conservation concern, whereas others have become overabundant in many regions. Reliable data on deer population sizes are lacking in most cases. Non-invasive genetic approaches are promising tools for wildlife management, population size estimation being one important application.

2. We developed and tested a non-invasive genetic approach for red deer population estimation based on faeces collected from a free ranging red deer population in south western Germany. 1128 faeces samples were collected in a forested study area of 100 km², where increasing harvest rates in combination with unacceptable levels of browsing damage indicate a considerable population increase.

3. Genetic analysis of the samples yielded 398 reliable consensus genotypes. We determined the rate of misidentification due to genotyping error by conducting two different blind tests and calculated population size estimates for both sexes separately using the programs MARK and CAPWIRE. To account for closure violation, we augmented the transect grid by a seasonal male red deer home range radius when calculating population density.

4. A comparison of the resulting population densities to the red deer harvest in the study area shows that the harvest quota stipulated for the study area is too low to keep the population on a sustainable level. In further research, the issues of population closure and differences between the sexes in the detection probability should be addressed.

5. *Synthesis and Applications*. The presented population estimation approach can serve as a valuable tool for the management of deer populations. It allows sex specific assessment of population size, which is particularly useful for dimorphic species like the red deer.

[5] Corresponding publication: Ebert, C., Marell, R., Rahlfs, M., Spielberger, B., Hohmann, U.: Estimating red deer (*Cervus elaphus*) population size based on non-invasive genetic sampling. Submitted to the *European Journal of Wildlife Research*.

Keywords: Capture-mark-recapture, genotyping, misidentification, sexual dimorphism, population closure, harvest

Introduction

For any form of population monitoring and management, the assessment of population size and population changes is essential (Smart et al. 2004). This is true for rare or endangered populations, but also for abundant animals which can severely impact their environment (Jacob et al. 2009; Gordon et al. 2004). For different deer species, like for other large ungulates, these two extremes exist. Some deer species or populations are endangered and of conservation concern, whereas other are overabundant (Barrio 2007, Haji et al. 2008). High densities of deer can cause considerable damage through e.g. bark stripping or browsing and have an impact on vegetation composition and species richness (Allombert et al. 2005; Putman & Moore 1998). In many parts of Europe, red deer densities have increased (Milner et al. 2006; Mysterud et al. 2007). In such cases, an effective management of deer populations is necessary to limit impacts on the environment (Ward 2005). However, estimating population size for red deer is a difficult task. Deer populations in forested areas are particularly difficult to survey, because direct counts are not feasible and indirect methods like e.g. pellet counts yield imprecise results (reviewed in Smart et al. 2004). Thus for many regions in Europe reliable census data are not available, even though they are crucial in order to establish efficient and sustainable management plans as well as for conservation in case of endangered populations (Milner et al. 2006).

Non-invasive genetic approaches represent a powerful tool for population estimation of animals that are elusive and difficult to survey (Woods et al. 1999; Beja-Pereira et al. 2009). DNA contained in hair or faeces samples can be used for identification of individual animals, and these data can be used for population estimation e.g. implemented in a capture-mark-recapture (CMR) framework. CMR can yield absolute and precise population estimates (Otis et al. 1978; Seber 1982, Pollock et al. 1990), and non-invasive genetic CMR offers the advantage that animals do not have to be captured physically, but are detected through their genotype. This can reduce some sources of bias which are problematic in traditional CMR (McKelvey & Schwartz

2004; Petit & Valière 2006). However, several issues remain which can compromise non-invasive population estimates and thus have to be carefully taken into account when conducting a study (Ebert et al. 2010). Detection probabilities may be heterogeneous among individuals or vary over time, and violation of the assumption of population closure can exacerbate the definition of the studied population and assessment of population density (Boulanger & McLellan 2001; Boulanger et al. 2004a). Markers used to discriminate individuals have to be sufficiently informative, otherwise a genotype may not represent a unique 'mark' (Pompanon et al. 2005). Genotyping errors such as allelic dropout or false alleles can lead to misidentification of individuals and thus to overestimation of population sizes (Prugh et al. 2005; Waits & Paetkau 2005). Therefore, care must be taken to reduce genotyping errors as far as possible e.g. by repeating analyses several times and by using effective error-checking protocols (Broquet & Petit 2004; McKelvey & Schwartz 2004). Techniques for non-invasive monitoring based on faeces samples have been tested for several ungulates, e.g. Alpine ibex (*Capra ibex*; Hausknecht et al. 2009). However, studies in which a non-invasive population estimation method is de facto developed and applied are rare for ungulates (but see Harris et al. 2009). For red deer, a first evaluation of the technical background for non-invasive population monitoring has been carried out in a pilot study (Valière et al. 2006), but until now to our knowledge a population estimation study in the field has not been realised for this species.

In this study, we established a non-invasive genetic population estimation method based on faeces samples collected along transect lines in a free ranging red deer population in south western Germany. In most federal states of Germany, red deer populations are restricted to assigned, mostly forested regions, habitat patches thus being highly fragmented. About 23% of the countries total area is deer habitat (Kinser, Koop & von Münchhausen 2010). Populations are in general harvested, but reliable census data which would allow the evaluation of management measures and validation of the harvest quotas are lacking.

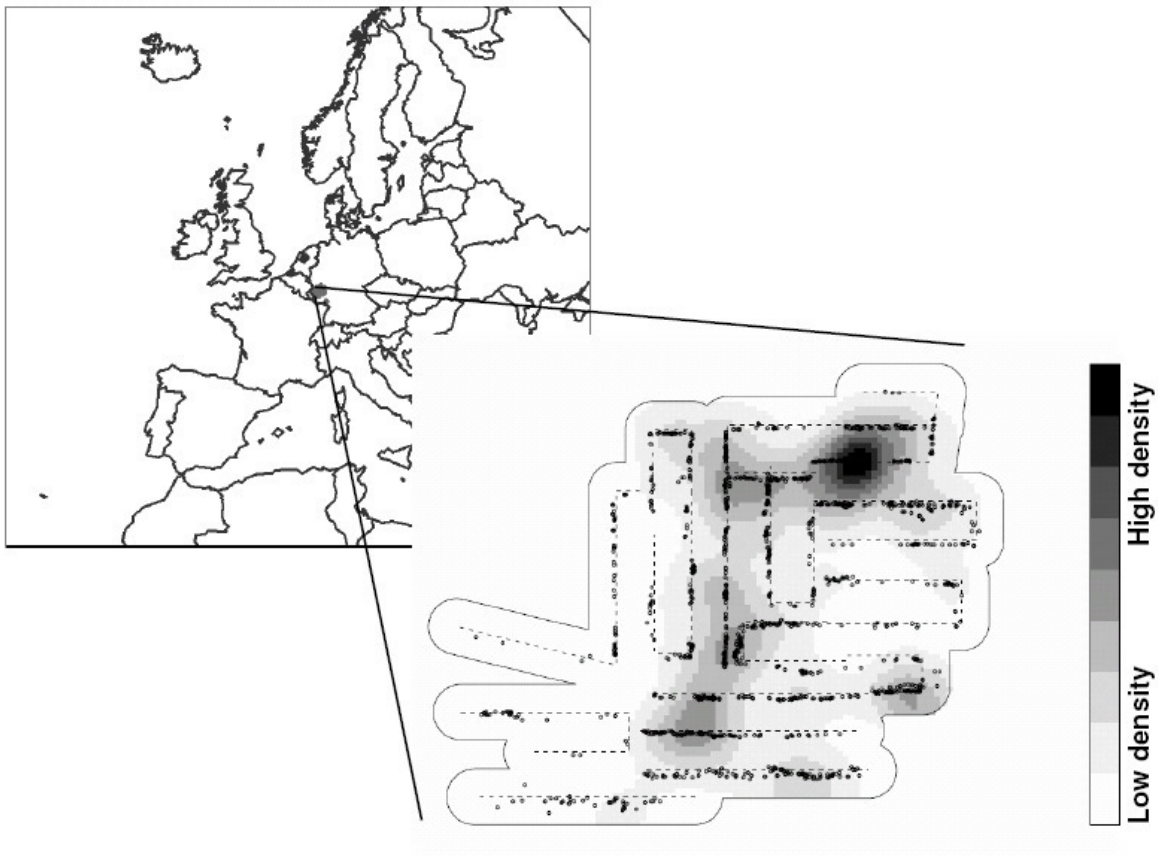

Figure 1: Location of the study area in south western Germany and overview over the transect grid. Dashed lines represent transects, black dots indicate locations of faeces samples. The buffer around the transect grid represents the effectively sampled area which consists of the transect grid including a buffer of 750 m, corresponding to a mean seasonal home range radius of red deer stags in the neighbouring Vosges. Areas shaded in different intensities show the distribution of faeces in the area, dark regions represent hot spots where most samples have been found.

Material and methods

STUDY AREA AND FIELD SAMPLING

Faeces were collected in a 100 km²- study area situated in the Palatinate Forest in the federal state of Rhineland-Palatinate, south western Germany (49°12'N, 7°45' E). Elevation ranges from 210 m to 609 m a.s.l. The area is covered with forest to approximately 90%, with beech forest (Luzulo-Fagetum) as predominant native plant community. 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*). Red deer are hunted during drive hunts between mid-October and the end of January. Furthermore, single hunt from raised stands is carried out from June 1st (fawns) and August 1st (stags and hinds) respectively until December 31st. Game harvest in the study area is state-run. The average red deer hunting bag from 1999 to 2009 is 1.0 per km² and year (minimum 0.7, maximum 1.3).

For collection of red deer faeces, we established 16 transects with a total length of 111 km, single transect length varying between 4.2 and 9.7 km. We defined the distance between transects to be ≤ 1 km, corresponding to the mean diameter of daily home ranges determined for hinds in the neighbouring French Vosges (Hamann et al. 1997). Faeces were collected between the 15th and 26th March 2010, each transect was searched daily for 10 days with a break of two days between day 5 and 6. The locations of all detected red deer faeces were recorded using GPS loggers (Mobile Action Inc., I-gotU GT 120, http://www.i-gotu.com).

In order to increase the genotyping success rate, only sufficiently fresh faeces (i.e. intact pellets with moist, shiny surface) were collected for analysis. Approximately one hand full of pellets of each fresh pellet group was collected using an inverted freezer bag which was then reversed and closed. Samples were stored frozen (-19ºC) in the sealed freezer bags until analysis.

DNA EXTRACTION AND GENOTYPING

For isolation of DNA from faeces samples, a commercial kit (Chemagen chemagic stool kit, Baesweiler, Germany) was used. To achieve a high proportion of target DNA, the standard protocol was modified in that two pellets of each sample were incubated with 3 ml lysis-buffer at room temperature in a 50 ml Falcontube, thereby avoiding the destruction of the pellets.

For a quality pre-screening, we determined the amount of target DNA in each sample after extraction by real-time PCR (qPCR). This approach allows an accurate determination of usable DNA per sample regardless of the total amount of DNA (Beja-Pereira et al. 2009). We established a qPCR on transgelin as a single copy gene (Acc. No. DQ12697 Cervus elaphus transgelin mRNA) to determine the copy number of target DNA. We defined a cycle threshold of 0.05 ng target DNA to exclude low quality samples from further analysis.

For the selection of appropriate microsatellite markers in red deer, we analysed in a pilot study isolated DNA from 40 tissue samples using 16 markers (data not shown). Of these, we selected seven dinucleotide markers for further analyses. We carried out polymerase chain reaction (PCR) amplification of the amelogenin gene according

to Gurgul et al. (2010) for sex determination. The selected markers were combined and co-amplified in three separate multiplex PCR's. The thermocycling profile 95°C fifteen minutes was followed by 45 cycles of 94°C 30 seconds, 57°C 90 seconds, and 72°C 60 seconds, then 60°C 30 minutes. Amplification reactions were performed in triplicates in a total volume of 12 µl using the Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany). The primers were used at concentrations of 0.075 µM to 0.3 µM. We separated fluorescently labelled DNA fragments on an ABI3730 DNA analyser and determined allele size using the ABI GS500LIZ size ladder (Applied Biosystems, Darmstadt, Germany).

We deduced consensus genotypes from the triplicate results. We typed samples as heterozygous at one locus if both alleles appeared at least twice, and as homozygous when all replicates showed the same result. We repeated the genotyping another three times when results were ambiguous after the first three replicates. We discarded all samples which failed to amplify or to produce unambiguous results for more than two loci.

Determination of matching genotypes and construction of capture histories were carried out using GENECAP (Wilberg & Dreher 2004). In order to confirm the power of the used loci, we calculated the probability of identity (PID) and, being more conservative, PID for siblings (Waits et al. 2001) using GIMLET (Valière 2002). We furthermore used GIMLET to calculate genotyping error rates (allelic dropout [ADO] and false alleles [FA]) and heterozygosity.

For population estimation, not only the genotyping error rates are relevant, but also how often whole genotypes are incorrectly identified. Thus, we additionally estimated a misidentification rate (i.e. false identification of a whole sample due to genotyping errors, leading to an erroneous genotype) based on two blind tests. For the first, a second sample was taken of 50 of the red deer faeces and analysed together with all the other samples, without the lab personnel having knowledge about which samples were duplicates. The percentage of duplicate samples that did not match their corresponding original sample was defined to represent the misidentification rate. For the second blind test we used faeces from 13 captive red deer. These samples were collected immediately after defecation under direct observation, so that for each

sample the defecating individual and its sex and age class could be recorded. Each of the samples was divided into 2 to 6 subsamples yielding a total of 40 subsamples, which were numbered randomly and then sent into the lab for analysis.

Consensus genotypes were obtained for both original and duplicate for 19 of the 50 pairs of samples in the first blind test. Of these, 18 pairs were found to match, and one pair showed differences in two loci as well in the sex marker and was thus considered as a mismatch, resulting in a misidentification rate of 5%. In the second blind test based on samples from captive deer, 34 subsamples yielded usable consensus genotypes. All 34 subsamples were assigned correctly to the right individual, thus in this test no misidentifications occurred.

For purpose of validation for the faeces genotyping, we collected tissue samples from 44 red deer harvested in the study area after the faeces sampling trial until the end of our study (April to November 2010). We isolated DNA from tissue samples using the chemagic tissue kit (Chemagen Baesweiler, Germany) and following the manufacturers' instructions. Tissue samples were genotyped using the same set of markers as used for the faeces samples.

POPULATION ESTIMATION

Population estimates were calculated using full closed capture models in program MARK (White & Burnham 2001). We included sex as a grouping variable and defined a set of 36 candidate models which were then compared using Akaike's Information Criterion with an additional bias correction term (AICc) in order to determine which models were most supported by the data (Burnham & Anderson 2002). The candidate models are deduced from six basic models for which we chose nomenclature according to Otis et al. (1978):

- M 0 (Null) with constant capture probability (p)
- M t with p varying over time
- M b with behavioural response to capture and thus recapture probability c different from p
- M h, a mixture model accounting for individual heterogeneity (IH)
- M th with p varying over time plus IH

- M bh with behavioural response and IH

We considered each of these basic models in a sex- specific form and with parameters equal for both sexes. From each of these 12 different possibilities, we built 3 models which varied according to their handling of misidentification. For the first, we fixed the identification parameter alpha to 1 (no misidentification, according to blind test 2), for the second, we set alpha = 0.95 (5% misidentification, according to blind test 1), and in the third alpha was an estimated parameter.

We calculated model averages, i.e. weighted averages over all models according to their support in the data as indexed by the AICc weights, in order to account for model selection uncertainty (Burnham & Anderson 2002). Since in program MARK, confidence intervals (CI) for model averages do not account for the minimum number of observed individuals, we calculated CI using the unconditional standard error (SE) and the equation reported in Rexstad & Burnham (1992; page 19).

Additionally, we calculated population sizes using CAPWIRE, (Miller et al. 2005). This program has been designed for use with non-invasive data sets. It uses a continuous sampling approach and allows taking into account all detections of an animal, including multiple detections in the same sampling session. We used separate data sets for each sex in CAPWIRE to account for potential differences between the sexes.

Since there are no natural boundaries to the north and east of our study area, we tested the assumption of population closure. We consider the population as demographically closed since we conducted our sampling well out of the farrowing and the hunting season. To examine geographic closure, we used Pradel models for open populations in MARK (Boulanger & McLellan 2001). These incorporate apparent survival (Φ) and recruitment (f), which we constrained in order to create closed or partly closed populations. Setting Φ = 1 represented no losses in the population, and setting f = 0 represented no additions. We built different models without constraints or with partial constraints only on one of the sexes and/ or only on Φ or f. These models each were compared to the completely constrained model (Φ and f for both sexes closed) using a likelihood ratio test.

Table 1: Characteristics of the microsatellite markers used for individual identification of red deer faeces samples (H exp = expected heterozygosity, H obs = observed heterozygosity, PCR+ = positive PCR, ADO = allelic dropout, FA = false allele).

Marker	Reference	No. of alleles	H exp	H obs	PCR+	ADO	FA
Haut14	Kühn et al. 2003	11	0.81	0.82	0.92	0.04	0.010
CSSM16	Kühn et al. 2003	8	0.76	0.77	0.93	0.05	0.000
BM203	Valière et al. 2006	13	0.89	0.81	0.92	0.07	0.000
CSSM19	Kühn et al. 2003	14	0.87	0.84	0.90	0.08	0.000
BMC1009	Valière et al. 2006	12	0.87	0.86	0.90	0.07	0.005
TGLA53	Valière et al. 2006	14	0.69	0.54	0.88	0.03	0.000
IDVGA55	Valière et al. 2006	8	0.76	0.75	0.90	0.07	0.000
Mean		11.4	0.81	0.77	0.91	0.06	0.002

Results

FIELD SAMPLING AND GENOTYPING

During the ten sampling days, a total of 2.239 red deer faeces were detected. Of these, 1.128 were considered as fresh enough for genotyping and were thus collected. Of the 1.128 samples for which qPCR was carried out, 518 contained sufficient target DNA and were therefore genotyped. Usable consensus genotypes were obtained from 398 of these samples. The proportion of positive PCR varied among loci from 88% to 93%. The estimated PID over all loci was 2.226×10^{-10} and PIDsibs 4.570×10^{-04}. Mean expected heterozygosity (H exp) was 0.81 and mean observed heterozygosity (H obs) was 0.77 (Table 1). Significant differences between H exp and H obs occurred in only one locus (TGLA 53). The mean ADO rate estimated from all samples over all markers was 6%, whereas the FA rate averaged 0.2%.

Table 2: Capture frequencies of 247 individual red deer detected via faeces sampling in the Palatinate Forest. 'Recaptures' stands for detections of the same individual on different days (corresponding to the recaptures in CMR), 'all detections' represents all samples found from the same individuals, thus the recaptures plus detections on the same day as used for population estimation via CAPWIRE.

No. of captures per individual	Capture frequencies recaptures		Capture frequencies all detections	
	Male	Female	Male	Female
1	60	106	57	105
2	31	27	27	26
3	9	8	12	8
4	0	1	4	3
5	4	0	2	0
6	0	0	1	0
7	0	0	1	0
8	1	0	1	0

The 398 observed genotypes corresponded to 247 different red deer individuals, of which 105 were male and 142 were female (sex ratio 1 : 1.35). Genotypes were observed in mean 1.85 times (males) and 1.36 times (females). While 54% of all detected male red deer were sampled more than once, this was the case for only 26% of the females (Table 2).

POPULATION ESTIMATION

Four of the MARK candidate models were supported by the data (indicated by ΔAICc ≤ 2), the most supported models incorporating IH and time dependence in p (Table 3). The model averaged population estimate indicates a population of 161 (126 – 252) male and 249 (174 – 495) female red deer, the estimated sex ratio therefore being 1 : 1.55 (Table 3). The average estimated per-session capture probability was 0.1 (0.13 for males and 0.07 for females).

Table 3: Overview over the ten highest ranked candidate models for non-invasive population estimation of red deer in the Palatinate Forest. Model selection is based on Akaike's Information Criterion corrected for small sample sizes (AICc). Further parameters given are K (number of parameters), w_i (model weights), N (estimated population size) and standard error (SE) for both sexes including 95 % confidence intervals (mis = misidentification). For details of model nomenclature see text.

Model	K	Δ AICc	w_i	$\hat{N}$ male	SE	95% CI	$\hat{N}$ female	SE	95% CI
M th 5% mis	23	0.000	0.334	164	13.8	139 - 194	222	17.4	191 - 259
M th	23	0.198	0.302	178	15.3	150 - 210	241	19.2	206 - 281
M th sex mis estimated	45	0.925	0.210	135	36.3	81 - 227	317	121.5	153 - 655
M th mis estimated	24	2.013	0.122	158	41.1	95 - 260	213	55	129 - 351
M th sex	44	4.751	0.031	152	13.1	128 - 180	289	34.5	229 - 365
M t sex mis estimated	25	14.592	0.000	93	14.6	69 - 127	178	35.2	121 - 261
M t sex 5% mis	24	16.644	0.000	129	8.7	113 - 148	256	28.3	206 - 318
M t sex	24	18.468	0.000	140	9.8	122 - 160	277	31.1	223 - 345
M t mis estimated	14	21.794	0.000	99	18.2	70 - 142	135	24.4	95 - 192
M th sex 5% mis	44	24.135	0.000	145	13.5	121 - 174	256	28.3	206 - 317
Model average				161	29.3	126 - 252	249	71.8	174 - 495

Table 4: Support for Pradel open population models of a red deer population in the Palatinate Forest. Survival (Φ) and recruitment (f) were constrained to create closed or partially closed models (details see text). Models are shown in pairs, each of the open or partially open models is compared (in descending order according to their model fit and weight) with the model in which Φ and f are closed for both sexes. The parameters shown are Akaikes Information Criterion corrected for small sample sizes (AICc), number of parameters (K), model weight (w_i), and as results of the likelihood tests the X^2 value, degrees of freedom (df, here the difference in K between a pair of models), and the probability of obtaining a X^2 value equal or larger when there is no difference in model fit.

Model	AICc	K	w_i	X^2	df	*P*
Φ M+F open, f M+F closed	1787.27	4	0.465	116.37	2	< 0.0001
Φ + f both sexes closed	1899.55	2	0.000			
Φ M+F open, f M closed, F open	1788.35	5	0.271	117.34	2	< 0.0001
Φ + f both sexes closed	1899.55	2	0.000			
Φ M+F open, f M open, F closed	1789.32	5	0.167	116.37	3	< 0.0001
Φ + f both sexes closed	1899.55	2	0.000			
Φ + f both sexes open	1790.41	6	0.097	117.35	4	< 0.0001
Φ + f both sexes closed	1899.55	2	0.000			
Φ + f M closed, F open	1830.20	4	0.000	73.53	2	< 0.0001
Φ + f both sexes closed	1899.55	2	0.000			
Φ + f M open, F closed	1859.81	4	0.000	43.83	2	< 0.0001
Φ + f both sexes closed	1899.55	2	0.000			
Φ M+F open, f M closed, F open	1788.35	5	0.271	0.98	1	0.3230
Φ M+F open, f M+F closed	1787.27	4	0.465			
Φ M+F open, f M open, F closed	1789.32	5	0.167	0.006	1	0.9305
Φ M+F open, f M+F closed	1787.27	4	0.465			
Φ + f both sexes open	1790.41	6	0.097	0.982	2	0.6119
Φ M+F open, f M+F closed	1787.27	4	0.465			

CAPWIRE yielded estimates of 194 (141 – 216) male and 389 (287 – 444) female red deer. For both sexes, the two innate rates model (TIRM) was chosen as appropriate in the program-inherent likelihood ratio test in favour of the equal catchability model (ECM).

All supported Pradel models showed an unconstrained (i.e. open) Φ for both sexes, the most supported model having constrained (i.e. closed) f. Models with open f for one or both sexes were less supported, but still received considerable support (Table 4). Differences in model fit were significant for all of the open or partly open models versus the completely closed model. In contrast, the open and partly open models did not differ significantly among each other concerning their GOF.

Because the results of the Pradel modelling indicate that the assumption of population closure is not met, we used an approximation of the effectively sampled area (ESA, Tioli et al. 2009) to calculate population density. Therefore, we added a buffer around the transect grid. We calculated the buffer using the radius of a mean male red deer seasonal home range (16.8 km² ; Klein & Hamann 1997), resulting in a buffer width of 750 m. The ESA consequently covers a total of 129 km², which is 22.5 % more than the transect grid area.

The population density derived from the MARK model average is 1.24 (0.98 – 1.95) male and 1.92 (1.35 – 3.84) female red deer per km² (Figure 2). The CAPWIRE estimates yielded densities of 1.5 (1.09 – 1.67) male and 3.0 (2.22 – 3.44) female red deer per km².

After the end of the faeces sampling trial, tissue samples were collected from 44 harvested deer. Of these, 31 (14 males and 17 females) belonged to individuals older than 10 month which could thus have been present in the study area at the time of faeces sampling. Thirteen of these individuals (eight males and five females) represented genotypes which had been detected at least once in the faeces sampling. This corresponds to a 'recapture' rate between faeces sampling and harvest of 42%.

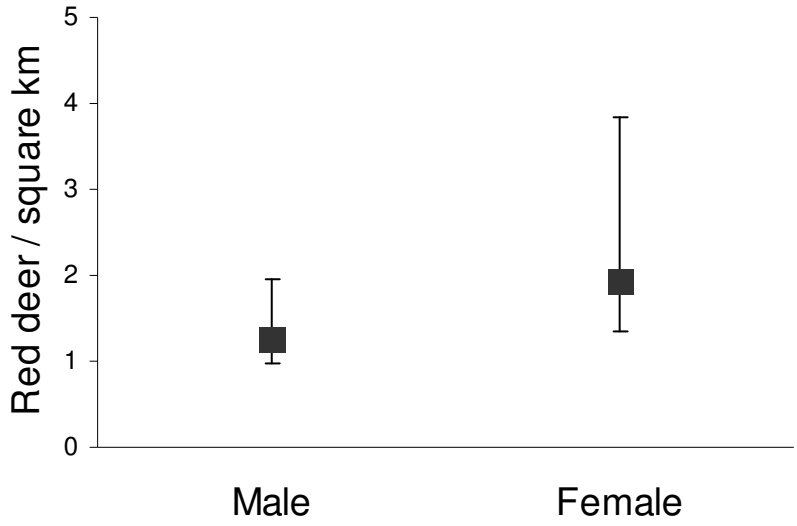

Figure 2: Estimated densities of male and female red deer in a study area situated in the Palatinate Forest. Density calculation was based on an effective sampling area of 129 km² (details see text). Bars represent 95% confidence intervals.

Discussion

Of the samples which were genotyped after the qPCR, 77% yielded an unambiguous consensus genotype. This indicates that pre-screening the samples by determining the concentration of target DNA was useful to maximise the output, since the success rate lies in the upper range compared to other studies (Broquet, Ménard & Petit 2007). PIDsibs being well below 0.01, we consider the set of seven loci as sufficient for discrimination between individuals. The overall genotyping error rate lies in the range of those reported in other studies (reviewed e.g. in Valière et al. 2006). By conducting three to six PCR replicates per sample, we were able to keep the total misidentification rate ≤ 5%, which can be considered as sufficient for the purpose of population estimation (Taberlet & Luikart 1999, Lukacs & Burnham 2005b). However, even for genotyping error rates in the range of 5%, population estimates can be considerably biased (Roon et al. 2005b). In a simulation study, Valière et al. (2006) showed that with residual errors of 1-3%, population estimates with a small relative bias (below 10%) can be obtained. The misidentification rate in our study ranges between those values.

For population estimation based on non-invasive genetic sampling, it has been recommended to collect 2.5 to 3 times as many samples as animals are assumed to be present in the population (Solberg et al. 2006). In our case, this has been achieved when considering the MARK model average – the ratio collected samples/ estimated N was 2.75. For CAPWIRE the ratio was 1.93. Miller, Joyce & Waits (2005) used the average number of observations per individual as measure for the sampling success and recommend that 2 to 2.5 observations per individual can yield estimates in the range of 15% to 10% from the real population size. In our case, males were closer to the recommended value (1.85 obs./ind.) than females (1.36 obs./ind.). Taking into account these different measures, it seems that the sample size in our study ranges at the lower bound of the values recommended in literature. Nevertheless, the number of identified genotypes represents 60% of the estimated population size (65% for males and 57% for females). This indicates that a large part of the population is represented in the faeces samples, which is corroborated by the fact that 42% of the red deer harvested in the months after sampling had been detected via faeces sampling.

The results of the MARK model selection procedure indicate that IH in capture probability is present in the sampled population in conjunction with variation over time. The latter most probably reflects a decrease in the number of samples collected over the 10 sampling days. It is possible that the field workers who walked the transects daily had disturbed the red deer and resulted in an avoidance of the disturbed areas. Neumann et al. (2010) showed that moose (*Alces alces*) reacted with short-term movements to humans skiing off-trail and left the area where they were disturbed. Red deer might also respond to even a single walking person with flight or displacement. It has been shown that flight distances of ungulates were greater when humans were walking off-trail, as was the case in our study, compared to humans hiking on trails (Stankowich 2008). However, in another faeces sampling trial we conducted in March 2009 using the same transects, we did not observe a distinct decrease over time in the number of samples collected per day. Furthermore, models accounting for behavioural response of the animals were not supported by the data, which we would have expected if the red deer had reacted to our activities during sampling. Nevertheless, we can not completely rule out the possibility that red deer – especially females, which in other ungulates tend to flee at greater distances when disturbed (Stankowich 2008) – reacted with displacement to humans walking off-trail during faeces sampling. This aspect requires further research, e.g. by observing red deer in the study area using GPS collars and monitoring their reaction to human presence.

Heterogeneity in capture probability is a problematic issue for population estimation in traditional and in non-invasive CMR approaches and can bias population estimates low (Otis et al. 1978; Boulanger et al. 2004a; Ebert et al. 2010). In our study, there was considerable variation in individual capture probabilities (visible in the capture frequencies, table 2), which could have been caused by individual differences in faecal DNA amount, defecation rates, habitat use, or territoriality (Bellemain et al. 2005; Lukacs & Burnham 2005b). In addition to IH, there was a considerable difference between the sexes in the mean capture probability. A larger proportion of the sampled males were captured multiple times compared to females, even if in total more females were detected. The hypothesis that females react stronger to disturbances than males and thus may have avoided the transect routes might be an explanation for this finding. However, the difference between the sexes may also

have been caused by larger male home ranges covering more transect lines (Hamann, Klein & Saint-Andrieux 1997, Klein & Hamann 1999, Nahlik et al. 2009). For sexually dimorphic species like the red deer, it is reasonable to treat the sexes separately for population estimation (Clutton-Brock & McLonergan 1994; Harris et al. 2010). Our results confirm this for red deer.

The point estimates generated using CAPWIRE corroborate the MARK estimates for the males, but indicate a higher population size for the females. CAPWIRE, in particular the TIRM, tends to overestimate population size for large N (Miller, Joyce & Waits 2005, Puechmaille & Petit 2007). Furthermore, it does not allow accounting for misidentification – which can also bias population sizes high. Thus, we believe the estimates derived using program MARK to be closer to reality. Additionally, the extremely female-biased sex ratio in the CAPWIRE estimate seems unlikely. Although in many ungulate populations sex ratios are female-biased (Clutton-Brock & McLonergan 1994), the sex ratio of the faeces sample (1 : 1.35) as well as that of the hunting bag in our study area (1 : 1.24) suggest a moderate excess of females in the studied population.

An approach to increase the sample size and to decrease individual and sex-based heterogeneity for red deer population estimation would be to sample the harvest in addition to faeces sampling (Dreher et al. 2007). The fact that 42% of the red deer harvested in our study area matched with genotypes found in the faeces sampling suggests that this approach is promising, even though the low total number of harvested deer prevented us from using the data to augment the population estimate. Population closure is a major concern in DNA mark-recapture studies like in traditional CMR (Boulanger & McLellan 2001). Closure violation can result in positive bias of population estimates, when animals move in and out of the sampling grid. The results of Pradel modelling in MARK indicate that there has been some closure violation. The fact that models with open Φ and completely or partly (i.e. for one of the two sexes) closed f received the most support suggests that movement out of the study area was predominant compared to movement into the area. It is possible that this result was caused by reactions of the animals towards human presence in their habitat (see above). The alternative use of open-population models suited for estimation of population size is not applicable for our data, since open models cannot

account for individual heterogeneity and their use requires larger sample sizes and longer study times compared to closed models (Pollock 1990, Boulanger & McLellan 2001, Luikart et al. 2010). However, population estimates can still be meaningful when closure is violated. In that case they can be considered as superpopulation estimates, i.e. the sampled population extends beyond the sampling grid boundaries (Roon et al. 2005b). Closure violation exacerbates the determination of population density. Nevertheless, population density, i.e. the relation to the area for which a given population estimate is valid, is needed for most management purposes (Wilson & Anderson 1985). 'Naïve' density estimates can be severely biased when the closure assumption is not met (Wilson & Anderson 1985). To reduce bias, we calculated density using an approximation of the ESA by adding a buffer around the transect grid. The buffer is based on telemetry data from red deer in a study area located near ours and with a similar habitat. In our case – as in all cases when ESA is arbitrarily defined - the estimated population densities have to be used with caution, even if the basis for the buffer width seems reasonable. However, since the harvest quota is set on a similar basis, we believe the comparison of both values for management to be valid in our study area.

MANAGEMENT IMPLICATIONS

DNA-based population estimates often considerably exceed those obtained with conventional methods, the latter tending to underestimate populations (Kendall et al. 2009). Non-invasive methods can therefore be a valuable tool to assess the effects of hunting on population size and to set harvest quotas (Luikart et al. 2010). The harvest quota which has been set for 2010 in our study area was 0.8 red deer per km² (G. Scheffler, Forestry of Hinterweidenthal, pers. comm.). Regarding our population estimates, it is apparent that this quota is too low when the aim is to keep the population on a sustainable level. Taking into account our estimated female population size and applying a reproductive output rate of 75% (Mueller & Mueller 2004), we would expect an output of 1.5 (MARK model average) and 2.3 (CAPWIRE) red deer per km² for our study area. Thus the recommended harvest quota will not suffice to stop a population increase, and we recommend that the red deer management and harvest plan for our study area should be revised.

The results of our study show that non-invasive genetic population estimation based on faeces is a promising tool for the management of red deer populations, allowing a quantitative evaluation of conservation and management measures. It can represent a method to obtain reliable data on red deer populations which have until now been lacking in many regions of Europe and North America.

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Conclusion and management implications

Concerning wildlife, conservation on the one side and management on the other side have gained importance in the last decades, i.a. due to increasing habitat loss or fragmentation and due to conflicts between the needs of humans and wildlife. For both conservation and management, knowledge about population size and other population parameters is highly important. Non-invasive genetic methods allow estimation of population size without capturing or killing animals, making them advantageous for rare or endangered species. They can however also be promising for abundant species because the application of non-invasive CMR can yield absolute population estimates, and some sources of bias can be reduced when animals are not physically captured.

Our tests indicate that non-invasive hair sampling using baited hair traps is not practicable for wild boar, at least for the purpose of genotype-based population estimation. However, this study shows that using faeces as a DNA source for non-invasive genetic population estimation is feasible for wild boar and red deer.

Nevertheless, several issues should be addressed in further studies. For wild boar, the sample size and population coverage should be increased to further improve the consistency and precision of the estimates. In case of the red deer, the sample size ranged around the lower bound of the values recommended in literature. Thus, a further increase in sample size would be advantageous and could be realised e.g. by additionally collecting tissue samples of the harvested animals. For red deer, it should furthermore be assessed if the presence of humans in their habitat during sampling affects their behaviour and therefore their detection probabilities. For both species, the issue of geographic population closure should be investigated in detail, e.g. using radio- or GPS- telemetric observation of the animals' movements. Furthermore, individual heterogeneity, which is almost ubiquitous in population estimation studies, is present also in our wild boar and red deer data. In addition to applying models that account for heterogeneity and – in case of red deer – separating the data for the two sexes, one or more different sampling strategies could

be tested as a supplement to the faeces sampling. This could reduce heterogeneity and increase sample size.

Furthermore, a certain amount of individual misidentification due to genotyping errors is most probably present in both the wild boar and the red deer data sets (as it is the case in the vast majority of other non-invasive genetic studies). Even though we applied careful error-checking protocols to keep the misidentification rate as low as possible, this might have biased the population estimates high. The misidentification rate was tested for both species and ranged at or below 5% in both cases, indicating that potential upward bias will be moderate. For the wild boar, I decided to use a very conservative population estimation approach to account for model selection uncertainties due to the small sample size and misidentification. However, an even stricter laboratory approach to detect and eliminate genotyping errors could further increase the accuracy of the population estimates. The discriminating power and the reliability of the genetic marker system could be increased e.g. by using Single Nucleotid Polymorphisms (SNP's) in addition to or instead of microsatellites for individual identification (Beja-Pereira et al. 2009). This could perhaps also reduce the costs for non-invasive population estimation, which would be advantageous for large-scale application of the method.

For both studied species, the non-invasive population estimate revealed that population sizes were considerably larger than previously assumed. In the study area, for wild boar the aim of the forestry in charge was to reduce population sizes – even before our population estimates indicated that populations are larger than expected. In the case of red deer, the aim was to keep the population size on a sustainable level and prevent a further population increase. Our study indicates that for both species the harvest as a management strategy does not suffice to prevent an increase in population size, not to mention a reduction of the population. Thus, the management plans for wild boar and red deer in the study area should be revised.

The population estimation approach presented in this study allows a quantitative evaluation of the success of management measures, which until now was not available for the study area, and can help to determine sustainable management plans and harvest quotas for the studied populations. It therefore represents a

promising tool for management and conservation, which could be adapted for other red deer and wild boar populations as well as for other ungulate species. Still, more research should be done to rule out some sources of bias and to optimise the genetic methods.

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References

Acevedo, P., Escudero, M.A., Munoz, R., Gortàzar, C. 2006: Factors affecting wild boar abundance across an environmental gradient in Spain. Acta Theriologica 51, 327-336.

Acevedo, P., Vicente, J., Höfle, U., Cassinello, J., Ruiz-Fons, F., Gortàzar, C. 2007: Estimation of wild boar relative abundance and aggregation: a novel method in epidemiological risk assessment. Epidemiology and Infection 135, 519-527.

Allombert, S., Stockton, S., Martin, J.-L. 2005: A natural experiment in the impact of overabundant deer on forest invertebrates. Conservation Biology 19, 1917-1929.

Arrendal, J., Vilà, C., Björklund, M. 2007: Reliability of noninvasive genetic census of otters compared to field censuses. Conservation Genetics 8, 1097-1107.

Artois, M., Depner, K.R., Guberti, V., Hars, J., Rossi, S., Rutili, D. 2002: Classical swine fever (hog cholera) in wild boar in Europe. Revue Scientifique et technique (Office international Epizootic) 21, 287-303.

Baber, D.W., Coblentz, B.E. 1986: Density, home range, habitat use, and reproduction in feral pigs on Santa Catalina Island. Journal of Mammalogy 67, 512-525.

Bailey, R.E., Putman, R.J. 1981: Estimation of fallow deer (*Dama dama*) populations from faecal accumulation. Journal of Applied Ecology 18, 697-702.

Barrio, J. 2007: Population viability analysis of the Taruka, *Hippocamelus antisensis* (D'Orbigny, 1834) (*Cervidae*) in southern Peru. Revista Peruana de Biologìa 14, 193-200.

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]

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 Journal of Zoology 59, 193-214.

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 kreftii*) by genetic analysis of non-invasively collected material. Animal Conservation 6, 101-107.

Bates, D., Maechler, M. 2010: lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-33. http://CRAN.R-project.org/package=lme4.

Baubet, E. 1998: Biologie du sanglier en montagne: biodémographie, occupation de l'espace et régime alimentaire. Dissertation, University of Lyon [in French].

Beja-Pereira, A., Oliveira, R., Alves, P.C., Schwartz, M.K., Luikart, G. 2009: Advancing ecological understandings through technological transformations in non-invasive genetics. Molecular Ecology Resources 9, 1279-1301.

Belant, J.L., Van Stappen, J.F., Paetkau, D. 2005: American black bear population size and genetic diversity at Apostle Islands National Lakeshore. Ursus 16, 85-92.

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. Conservation Biology 19, 150-161.

Bellemain, E., Nawaz, M.A., Valentini, A., Swenson, J.E., Taberlet, P. 2007: Genetic tracking of brown bear in northern Pakistan and implications for conservation. Biological Conservation 134, 537-547.

Bieber, C., Ruf, T. 2005: Population dynamics in wild boar *Sus scrofa*: ecology, elasticity of growth rate and implications for management of pulsed resource consumers. Journal of Applied Ecology 42, 1203-1213.

Boersen, M.R., Clark, J.D. & King, T.L. 2003: Estimating black bear population density and genetic diversity at Tensas River, Louisiana using microsatellite DNA markers. Wildlife Society Bulletin 31, 197-207.

Boitani, L., Trapanese, P., Mattei, L. 1995a: Methods of population estimates of a hunted wild boar (*Sus scrofa* L.) population in Tuscany, Italy. IBEX Journal of Mountain Ecology 3, 204-208.

Boitani, L., Trapanese, P., Mattei, L., Nonis, D. 1995b: Demography of a wild boar (*Sus scrofa*, L.) population in Tuscany, Italy. Gibier Faune Sauvage, 12, 109-132.

Borchers, D.L., Buckland, S.T., Zucchini, W. 2002: Estimating Animal Abundance – Closed Populations. Statistics for Biology and Health, Springer, London, 314 pp.

Boulanger, J., McLellan. B. 2001: Closure violation in DNA-based capture-recapture estimation of grizzly bear populations. Canadian Journal of Zoology 79, 642-651.

Boulanger, J., White, G.C., McLellan, B., Woods, J., Proctor, M., Himmer, S. 2002: A meta-analysis of grizzly bear DNA capture-recapture projects in British Columbia, Canada. Ursus 13, 137-152.

Boulanger, J., Himmer, S., Swan, C. 2004: Monitoring of grizzly bear population trends and demography using DNA capture-recapture methods in the Owikeno Lake area of British Columbia. Canadian Journal of Zoology 82, 1267-1277.

Boulanger, J., Stenhouse, G., Munro, R. 2004a: Sources of heterogeneity bias when DNA capture-recapture sampling methods are applied to grizzly bear (Ursus arctos) populations. Journal of Mammalogy 85, 618-624.

Boulanger, J., McLellan, B.N., Woods, J.G., Proctor, M.F., Strobeck, C. 2004b: Sampling design and bias in DNA- based capture-capture-recapture population and density estimates of grizzly bears. Journal of Wildlife Management 68, 457-469.

Boulanger, J., Proctor, M., Himmer, S., Stenhouse, G., Paetkau, D., Cranston, J. 2006: An empirical test for DNA capture-recapture sampling strategies for grizzly bears. Ursus 17, 149-158.

Boulanger, J., Kendall, K.C., Stetz, J.C., Roon, D.A., Waits, L.P., Paetkau, D. 2008: Multiple data sources improve DNA-based capture-recapture estimates of grizzly bears. Ecological Applications 18, 577-589.

Briedermann, L. 1990: Schwarzwild. VEB Landwirtschaftsverlag, Berlin [in German].

Broquet, T., Petit, E. 2004: Quantifying genotyping errors in non-invasive population genetics. Molecular Ecology 13, 3601-3608.

Broquet, T., Ménard, N., Petit, E. 2007: Non-invasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. Conservation Genetics 8, 249-260.

Burnham, K.P., Anderson, D.R. 2002: Model selection and multimodel inference: A practical information-theoretic approach. 2nd edition. Springer Verlag, New York.

Burnham, K.P., Overton, W.S. 1979: Robust estimation of population size when capture probabilities vary among animals. Ecology 60, 927-936.

Cahill, S., Llimona, F., Gràcia, J. 2003: Spacing and nocturnal activity of wild boar *Sus scrofa* in a Mediterranean metropolitan park. Wildlife Biology 9, 3-13.

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

Chao, A.L., Jeng, S.L. 1992: Estimating population size for capture-recapture data when capture probabilities vary by time and individual animal. Biometrics 48, 201-216.

Choquet, R., Lebreton, J.-D., Gimenez, O., Reboulet, A.-M., Pradel, R. 2009: U-CARE: Utilities for performing goodness of fit tests and manipulating CApture-REcapture data. Ecography 32, 1071-1074.

Clutton-Brock, T.H., McLonergan, M.E. 1994: Culling regimes and sex ratio biases in highland red deer. Journal of Applied Ecology 31, 521-527.

Coleman, K., Wilson, D.S. 1998: Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. Animal Behaviour 56, 927-936.

Creel, S., Spong, G., Sands, J.L., Rotella, J., Zeigle, J., Joe, L., Murphy, K.M., Smith, D. 2003: Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. Molecular Ecology 12, 2003-2009.

Crespin, L., Choquet, R., Lima, M., Merritt, J., Pradel, R. 2008: Is heterogeneity of catchability in capture-recapture studies a mere sampling artefact or a biologically relevant feature of the population? Population Ecology 50, 247-256.

Cubaynes, S., Pradel, R., Choquet, R., Duchamp, C., Gaillard, J.-M., Lebreton, J.-D., Marboutin, E., Miquel, C., Reboulet, A.-M., Poillot, C., Taberlet, P., Gimenez, O. 2010: Importance of accounting for detection heterogeneity when estimating abundance: the case of French wolves. Conservation Biology 24, 621-626.

Dardaillon, M., Beugnon, G. 1987: The Influence of some environmental characteristics on the movements of wild boar *sus scrofa*. Biology Behaviour 12, 82-92.

Dingemanse, N.J., Both, C., van Noordwijk, A.J., Rutten, A.L., Drent, P. 2003: Natal dispersal and personalities in great tits (*Parus major*). Proceedings of the Royal Society (London) 270, 741-747.

Dreher, B.P., Winterstein, S.R., Scribner, K.T., Lukacs, P.M., Etter, D.R., Rosa, G.J.M., Lopez, V.A., Libants. S., Filcek, K.B. 2007: Non-invasive estimation of black bear abundance incorporating genotyping errors and harvested bear. Journal of Wildlife Management 71, 2684-2693.

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 zur 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., Kolodziej, K., Schikora, T.F., Schulz, H.K., Hohmann, U. 2009: 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. Acta Silvatica et Lignaria Hungarica 5, 167-177.

Ebert, C., Huckschlag, D., Schulz, H.K., Hohmann, U. 2010: Can hair traps sample wild boar (*Sus scrofa*) randomly for the purpose of non-invasive population estimation? European Journal of Wildlife Research 56, 583-590.

Ebert, C., Knauer, F., Storch, I., Hohmann, U. 2010: Individual heterogeneity as a pitfall in population estimates based on non-invasive genetic sampling: a review and recommendations. Wildlife Biology 16, 225-240.

Eggert, L.S., Eggert, J.A., Woodruff, D.S. 2003: Estimating population size for elusive animals: the forest elephants of Kakum National Park, Ghana. Molecular Ecology 12, 1389-1402.

Ellegren, H. 2004: Microsatellites: simple sequences with complex evolution. Nature Reviews 5, 435-445.

Fattebert, J., Fischer, C., Kleijer, A., Baubet, E., Daendliker, G. 2004: Wild boar population size estimation in the canton of Geneva. In: Bobek B, editor. 5th International Wild Boar and Suidae Symposium. Abstracts. Krakow, Poland.

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*). European Journal of Wildlife Research 52, 28-33.

Focardi, S., Isotti, R., Pelliccioni, E.R., Iannuzzo, D. 2002: The use of distance sampling and mark-resight to estimate the local density of wildlife populations. Environmetrics 13, 177-186.

Foran, D.R., Minta, S.C. & Heinemeyer, K.S. 1997: DNA-based analysis of hair to identify species and individuals for population research and monitoring.- Wildlife Society Bulletin 25: 840-847.

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. Molecular Ecology 12, 1649-1661.

Frantz, A.C., Schaul, M., Pope, L.C., Fack, F., Schley, L., Muller, C.P., Roper, T.J. 2004: Estimating population size by genotyping remotely plucked hair: the Eurasian badger. Journal of Applied Ecology 41, 985-995.

Gervasi, V., Ciucci, P., Boulanger, J., Posillico, M., Sulli, C., Focardi, S., Randi, E., Boitani, L. 2008: A preliminary estimate of the Apennine brown bear population size based on hair-snag sampling and multiple data source capture-recapture Huggins models. Ursus 19, 105-121.

Gethöffer, F., Sodeikat, G., Pohlmeyer, K. 2007: Reproductive patterns of wild boar (*Sus scrofa*) in three different parts of Germany. European Journal of Wildlife Research 53, 287-297.

Gordon, I.J., Hester, A.J., Festa-Bianchet, M. 2004: The management of wild large herbivores to meet economic, conservation and environmental objectives. Journal of Applied Ecology 41, 1021-1031.

Groot Bruinderink, G.W.T.A., Hazebroek, E. 1995: Modelling Carrying Capacity for wild boar *Sus scrofa* scrofa in a forest/heathland ecosystem. Wildlife Biology 1, 81-87.

Gurgul, A., Radko, A., Slota, E. 2010: Characteristics of X- and Y-chromosome specific regions of the amelogenin gene and a PCR-based method for sex identification in red deer (*Cervus elaphus*). Molecular Biology Reports 37, 2915-2918.

Guschanski, K., Vigilant, L., McNeilage, A., Gray, M., Kagoda, E., Robbins, M.M. 2009: Counting elusive animals: Comparing field and genetic census of the entire mountain gorilla population of Bwindi Impenetrable National Park, Uganda. Animal Conservation 142, 290-300.

Hahn, N., Eisfeld, D. 1998: Diet and habitat use of wild boar (*Sus scrofa*) in SW-Germany. Gibier Faune Sauvage, Game Wildlife 15, 595-606.

Haji, G.M., Charfi-Cheikrouha, F., Lorenzini, R., Vigne, J.-D., Hartl, G., Zachos, F.E. 2008: Phylogeography and founder effect of the endangered Corsican red deer (*Cervus elaphus corsicanus*). Biodiversity and Conservation 17, 659-673.

Hamann, J.L., Klein, F., Saint-Andrieux, C. 1997: Domaine vital diurne et déplacements des biches (*Cervus elaphus*) sur le secteur de La Petite Pierre (Bas-Rhin). Gibier Faune Sauvage 14, 1–17.

Harris, R.B., Winnie Jr., J., Amish, S.J., Beja-Pereira, A., Godinho, R., Costa, V., Luikart, G. 2010: Argali abundance in the Afghan Pamir using capture-recapture modelling from fecal DNA. Journal of Wildlife Management 74, 668-677.

Hausknecht, R., Salzer, K., Filli, F., Kuehn, R. 2009: Techniques for noninvasive genetic monitoring of Alpine ibex *Capra ibex*. Acta Theriologica 54, 277-287.

Hebeisen, C., Fattebert, J., Baubet, E., Fischer, C. 2008: Estimating wild boar (*Sus scrofa*) abundance and density using capture-resight methods with camera traps in conjunction with radio tracking. European Journal of Wildlife Research 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. Conservation Genetics 5, 405-410.

Hromas, J. 1996: Hog cholera in the Czech Republik. In: Proceedings of the Wild Boar Symposium 24-27.3.1996 in Sopron, Hungary, pp. 54-56.

Huckschlag, D. 2008: Development of a digital infrared video camera system for recording and remote capturing. European Journal of Wildlife Research 54, 651-655.

Ihaka, R., Gentleman, R. 1996: R: A language for data analysis and graphics. Journal of Computational and Graphical Statistics 5, 299-314.

Immell, D., Anthony, R.G. 2006: Estimation of black bear abundance using a discrete DNA sampling device. Journal of Wildlife Management 72, 324-330.

Jacob, G., Debrunner, R., Gugerli, F., Schmid, B., Bollmann, K. 2010: Field surveys of capercaillie (*Tetrao urogallus*) in the Swiss Alps underestimated local abundance of the species as revealed by genetic analyses of non-invasive samples. Conservation Genetics 11, 33-44.

Kaden, V. 1998: Klassische Schweinepest bei Wildschweinen - Vorkommen in Europa - Möglichkeiten der Seuchenverbreitung in der Schwarzwildpopulation. Amtstierärztlicher Dienst und Lebensmittelkontrolle 5 I/98: 62-67 [in German]

Kaden, V. 1999: Bekämpfung der klassischen Schweinepest beim Schwarzwild. Zeitschrift für Jagdwissenschaften 45, 45-49 [in German].

Kaminski, G., Brandt, S., Baubet, E., Baudoin, C. 2005: Life-history patterns in female wild boars (*Sus scrofa*): mother–daughter postweaning associations. Canadian Journal of Zoology 83, 474-480.

Kawarasaki, T., Kohsaka, T., Sone, M., Yoshida, M., Bamba, K. 1995: Detection of Y-bearing porcine spermatozoa by in-situ hybridization using digoxigenin-labeled, porcine male-specific DNA-probe produced by polymerase chain reaction. Molecular Reprododuction and Development 40, 455-459.

Kend444all, K.C., Stetz, J.B., Boulanger, J., Macleod, A.C., Paetkau, D., White, G.C. 2009: Demography and genetic structure of a recovering grizzly bear population. Journal of Wildlife Management 73, 3–17.

Keuling, O., Stier, N., Roth, M. 2007: Annual and seasonal space use of different age classes of female wild boar *Sus scrofa* L. European Journal of Wildlife Research 54, 403-412.

Keuling, O., Stier, N., Roth, M. 2008: How does hunting influence activity and spatial usage in wild boar *Sus scrofa* L.? European Journal of Wildlife Research 54, 729-737.

Kinser, A., Koop, K., von Münchhausen, H. 2010: Die Rotwildverbreitung in Deutschland. AFZ - Der Wald 5, 32-34 [in german].
456

Klein, F., Hamann, J.-L. 1999: Domaines vitaux diurnes et déplacements des cerfs mâles (*Cervus elaphus*) sur le secteur de la Petite Pierre (Bas-Rhin). Gibier Faune Sauvage, Game Wildlife 16, 251-271 [in French].

Knapp, S.H., Craig, B.A., Waits, L.P. 2007: Incorporating genotyping error into non-invasive DNA-based capture-recapture population estimates. Journal of Wildlife Management 73, 598-604.

Kohn, M.H., York, E.C., Kamradt, D.A., Haught, G., Sauvajot, R.M., Wayne, R.K. 1999: Estimating population size by genotyping faeces. Proceedings of the Royal Society (London) 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.

Kuehn, R., Schroeder, W., Pirchner, F., Rottmann, O. 2003: Genetic diversity, gene flow and drift in Bavarian red deer populations (*Cervus elaphus*). Conservation Genetics 4, 157–166.

Link, W.A. 2003: Nonidentifiability of population size from capture-recapture data with heterogeneous detection probabilities. Biometrics 59, 1123-1130.

Link, W.A. 2004: Individual heterogeneity and identifiability in capture-recapture models. Animal Biodiversity and Conservation 27, 87-91.

Long, R.A., Donovan, T.M., Mackay, P., Zielinski, W.J., Buzas, J.S. 2007: Comparing faeces detection dogs, cameras, and hair snares for surveying carnivores. Journal of Wildlife Management 71, 2018-2025.

Lowden, S., Finlayson, H., Macdonald, A., Downing, A., Goodman, S., Leus, S., Kaspe, K., Wahyuni, E., Archibald, A. 2002: Application of *Sus scrofa* microsatellite markers to wild suiformes. Conservation Genetics 3, 347-350.

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. Molecular Ecololgy 11, 857-868.

Luikart, G., Ryman, N., Tallmon, D.A., Schwartz, M.K., Allendorf, F.W. 2010: Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. Conservation Genetics 11, 355-373.

Lukacs, P.M., Burnham, K.P. 2005a: Review of capture-recapture methods applicable to non-invasive genetic sampling. Molecular Ecology 14, 3909-3919.

Lukacs, P.M., Burnham, K.P. 2005b Estimating population size from DNA-based closed capture-recapture data incorporating genotyping error. Journal of Wildlife Management 69, 396-403.

Maillard, D., Fournier, P., Fournier-Chambrillon, C. 1996: Influence of food availability and hunting on wild boar (*Sus scrofa* L.) home range size in a mediterranean habitat. In: Proceedings of the Wild Boar Symposium 24-27.3.1996 in Sopron, Hungary, pp. 69-81.

Massei, G., Cowan, D.P., Coats, J., Gladwell, F., Lane, J.E., Miller, L.A. 2008: Effect of the GnRH vaccine GonaCon on the fertility, physiology and behaviour of wild boar. Wildlife Research 35, 540-547.

Maudet, C., Luikart, G., Dubray, D., von Hardenberg, A., Taberlet, P. 2004: Low genotyping error rates in wild ungulate faeces sampled in winter. Molecular Ecology Notes 4, 772-775.

McKelvey, K.S., Pearson, D.E. 2001: Population estimation with sparse data: the role of estimators versus indices revisited. Canadian Journal of Zoology 79, 1754-1765.

McKelvey, K.S., Schwartz, M.K. 2004: Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. Journal of Wildlife Management 68: 439-448.

McLoughlin, P.D., Cluff, H.D., Gau, R.J., Mulders, R., Case, R., Messier, F. 2003: Effect of spatial differences in habitat on home ranges of grizzly bears. Écoscience 10, 11-16.

Meijer, T., Norén, K., Hellström, P., Dalén, L., Angerbjörn, A. 2008: Estimating population parameters in a threatened arctic fox population using molecular tracking and traditional field methods. Animal Conservation 11, 330-338.

Melis, C., Szafrańska, P.A., Jędrzejewska, B., Bartoń, K. 2006: Biogeographical variation in the population density of wild boar (*Sus scrofa*) in western Eurasia. Journal of Biogeography 33, 803-811.

Menkens, G.E., Anderson, S.H. 1988: Estimation of small-mammal population size. Ecology 69, 1952-1959.

Mettke-Hofmann, C., Ebert, C., Schmidt, T., Steiger, S., Stieb, S., 2005. Personality traits in resident and migratory warbler species. Behaviour 142, 1357-1375.

Miquel. C., Bellemain, E., Poillot, C., Bessière, J., Durand, A., Taberlet, P. 2006: Quality indexes to assess the reliability of genotypes in studies using non-invasive sampling and multiple-tube approach. Molecular Ecology Notes 6, 985-988.

Miller, C.R., Joyce, P., Waits, L.P. 2005: A new method for estimating the size of small populations from genetic capture-recapture data. Molecular Ecology 14, 1991-2005.

Mills, L.S., Citta, J.J., Lair, K.P., Schwartz, M.K., Tallmon, D.A. 2000: Estimating animal abundance using non-invasive DNA sampling: promise and pitfalls. Ecological Applications 10, 283-294.

Milner, J., Bonenfant, C., Mysterud, A., Gaillard, J.-M., Csányi, S., Stenseth, N.C. 2006: Temporal and spatial development of red deer harvesting in Europe: biological and cultural factors. Journal of Applied Ecology 43, 721-734.

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

Monaco, A., Franzetti, B., Pedroti, L., Silvano, T. 2004: Estimating wild boar population size: the inadequacy of “catch per unit effort” methods. In: Bobek B, editor. 5th International Wild Boar and Suidae Symposium. Abstracts. Krakow, Poland

Mowat, G., Paetkau, D. 2002: Estimating marten *Martes americana* population size using hair capture and genetic tagging. Wildlife Biology 8, 201-209.

Mowat, G., Strobeck, C. 2000: Estimating population size of Grizzly bears using hair capture, DNA profiling, and mark- recapture analysis. Journal of Wildlife Management 64, 183-193.

Mowat, G., Heard, D.C., Seip, D.R., Poole, K.G., Stenhouse, G., Paetkau, D.W. 2005: Grizzly *Ursus arctos* and black bear *Ursus americanus* densities in the interior mountains of North America. Wildlife Biology 11, 31-48.

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.

Mueller, F., Mueller, D.G. (editors) 2004: Wildbiologische Informationen für den Jäger. Band 1 Haarwild, 324 pp. [in German].

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. Conservation Genetics 8, 1219-1224.

Mysterud, A., Meisingset, E.L., Veiberg, V., Langvatn, R., Solberg, E., Loe L.E., Stenseth, N.C. 2007: Monitoring population size of red deer *Cervus elaphus*: an evaluation of two types of census data from Norway. Wildlife Biology 13, 285-298.

Neumann, W., Ericsson, G., Dettki, H. 2010: Does off-trail backcountry skiing disturb moose (*Alces alces*)? European Journal of Wildlife Research 56, 513-518.

Obbard, M.E., Howe, E.J., Kyle, C.J. 2010: Empirical comparison of density estimators for large carnivores. Journal of Applied Ecology 47, 76-84.

Otis, D.L., Burnham, K.P., White, G.C., Anderson, D.R. 1978: Statistical inference for capture-recapture experiments. Wildlife Monographs 62, 135 pp.

Paetkau, D. 2003: An empirical exploration of data quality in DNA- based population inventories. Molecular Ecology 12, 1375-1387.

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 carnivores: design and feasibility for the Iberian lynx. Molecular Ecology 11, 2171-2182.

Petit, E., Valière, N. 2006: Estimating population size with non-invasive capture-recapture data. Conservation Biology 20, 1062-1073.

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. Wildlife Research 30, 1-13.

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.

Pledger, S., Efford, M. 1998: Correction of bias due to heterogeneous capture probability in capture-recapture studies in open populations. Biometrics 54, 888-898.

Pollock, K.H., Nichols, J.D., Brownie, C., Hines, J.E. 1990: Statistical inference for capture-recapture experiments. Wildlife Monographs 107, 97 pp.

Pompanon, F., Bonin, A., Bellemain, E., Taberlet, P. 2005: Genotyping errors: causes, consequences and solutions. Nature Reviews Genetics 6, 847-859.

Poole, K.G., Mowat, G., Fear, D.A. 2001: DNA-based population estimate for grizzly bears *Ursus arctos* in northeastern British Columbia, Canada. Wildlife Biology 7, 105-115.

Pradel, R. 2005: Multievent, an extension of multistate capture-recapture models to uncertain states. Biometrics 61, 442-447.

Prigioni, C., Remonti, L., Balestrieri, A., Sgrosso, S., Priore, G., Mucci, N., Randi, E. 2006: Estimation of European otter (*Lutra lutra*) population size by fecal DNA typing in southern Italy. Journal of Mammalogy 87, 855-858.

Prugh, L.R., Ritland, C.E., Arthur, S.M., Krebs, C.J. 2005: Monitoring coyote population dynamics by genotyping faeces. Molecular Ecology 14, 1585-1596.

Puechmaille, S.J., Petit, E. 2007: Empirical evaluation of non-invasive capture-capture-recapture estimation of population size based on a single sampling session. Journal of Applied Ecology 44, 843-852.

Putman, R.J., Moore, N.P. 1998: Impact of deer in lowland Britain on agriculture, forestry and conservation habitats. Mammal Review 28, 141-164.

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. Conservation Genetics 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 [in German].

Rexstadt, E., Burnham, K.P. 1992: User's Guide for interactive program CAPTURE, abundance estimation of closed animal populations. Colorado Fish and Wildlife Research Unit, Colorado State University, Fort Collins CO, USA.

Rohrer, G.A., Alexander, L.J., Keele, J.W., Smith, T.P., Beattie C.W. 1994: A microsatellite linkage map of the porcine genome. Genetics 136, 231-245.

Roon, D.A., Thomas, M.E., Kendall, K.C., Waits, L.P. 2005a: Evaluating mixed samples as a source of error in non-invasive genetic studies using microsatellites. Molecular Ecology 14, 195-201.

Roon, D.A., Waits, L.P., Kendall, K.C. 2005b: A simulation test of the effectiveness of several methods for error-checking non-invasive genetic data. Animal Conservation 8, 203-215.

Rudnick, J.A., Katzner, T.E., Bragin, E.A., DeWoody, J.A. 2008: A non-invasive genetic evaluation of population size, natal philopatry, and roosting behaviour of non-breeding eastern imperial eagles (*Aquila heliaca*) in central Asia. Conservation Genetics 9, 667-676.

Ruis, M.A.W., te Brake, J.H.A., van de Burgwal, J.A., de Jong, I.C., Blokhuis, H.J., Koolhaas, J. 2000: Personalities in female domesticated pigs: behavioural and physiological indications. Applied Animal Behavioural Sciences 66, 31-47.

Sáez-Royuela, C., Telleria, J.L. 1986: The increased population of Wild Boar (*Sus scrofa* L.) in Europe. Mammal Review 16, 97-101.

Santini, A., Lucchini, V., Fabbri, E., Randi, E. 2007: Ageing and environmental factors affect PCR success in wolf (*Canis lupus*) excremental DNA samples. Molecular Ecology 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. Wildlife Biology 13, 195-207.

Schley, L., Dufrêne, M., Krier, A., Frantz, A.C. 2008: Patterns of crop damage by wild boar (*Sus scrofa*) in Luxemburg over a 10-year period. European Journal of Wildlife Research 54, 589-599.

Schnidig-Petrig, R., Koller, N. 2004: Praxishilfe Wildschweinmanagement. Service romand de vulgarisation agricole (SRVA), Lausanne, Schweiz.

Seber, G.A.F. 1982: The Estimation of Animal Abundance and Related Parameters. 2nd edition, Charles Griffin, London, 654 pp.

Seber, G.A.F. 1982: The estimation of animal abundance and related parameters. 2nd edition. Charles Griffin, London.

Servanty, S., Gaillard, J.-M.Toigo, C., Brandt, S., Baubet, E. 2009: Pulsed resources and climate-induced variation in the reproductive traits of wild boar under high hunting pressure. Journal of Animal Ecology 78, 1278-1290.

Settlage, K.E., Van Manen, F.T., Clark, J.D., King, T.L. 2008: Challenges of DNA-based capture-recapture studies of American black bears. Journal of Wildlife Management 72, 1035-1042.

Sloane, M.A., Sunnucks, P., Alpers, D., Behegaray, B., Taylor, A.C. 2000: Highly reliable genetic identification of individual northern hairy-nosed wombats from single remotely collected hairs: a feasible censusing method. Molecular Ecology 9, 1233-1240.

Smart, J.C.R., Ward, A., White, P.C.L. 2004: Monitoring woodland deer populations in the UK: an imprecise science. Mammal Review 34, 99-114.

Smith, D.A., Ralls, K., Cypher, B., Maldonado, J.E. 2005: Assessment of scat-detection dog surveys to determine kit fox distribution. Wildlife Society Bulletin 33, 897-904.

Sodeikat, G., Pohlmeyer, K. 2002: Temporary home range modifications of wild boar family groups (*Sus scrofa* L.) caused by drive hunts in Lower Saxony. European Journal of Wildlife Research 48, Suppl., 161-166.

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. Biological Conservation 128, 158-168.

Stankowich, T. 2008: Ungulate flight responses to human disturbance: A review and meta-analysis. Biological Conservation 141, 2159-2173.

Sweitzer, R., van Vuren, D., Gardner, I.A., Boyce, W., Waithman, J.D. 2000: Estimating sizes of wild pig Populations in the north and central coast regions of California. Journal of Wildlife Management 64, 531-543.

Taberlet, P., Luikart, G. 1999: Non-invasive genetic sampling and individual identification. Biological Journal of the Linnean Society 68, 41-55.

Taberlet, P., Waits, L.P., Luikart, G. 1999: Non-invasive genetic sampling: look before you leap. Trends in Ecology and Evolution 14: 323-327.

Thompson, W.L., White, G.C., Gowan, C. 1998: Monitoring Vertebrate Populations. Academic Press Inc., San Diego, 365 pp.

Thompson, S.K. 1991: Adaptive Cluster Sampling: designs with primary and secondary units. Biometrics 47, 1103-1115.

Tioli, S., Cagnacci, F., Stradiotto, A., Rizzoli, A. 2009: Edge effect on density estimates of a radiotracked population of yellow-necked mice. Journal of Wildlife Management 73, 184-190.

Tottewitz, F., Stubbe, Ahrens, M. , Dobiàs, K., Goretzki, J., Paustian, K.-H. 1998: Die Losungszählung als Methode der Bestandesschätzung von wiederkäuenden Schalenwildarten. Zeitschrift für Jagdwissenschaften 42, 111-122 [in german with english summary]

Toigo, C., Servanty, S., Gaillard, J.-M., Brandt, S., Baubet, E. 2008: Disentangling natural from hunting mortality in an intensively hunted wild boar population. Journal of Wildlife Management 72, 1532-1539.

Triant, D.A., Pace, R.M. III., Stine, M. 2004: Abundance, genetic diversity and conservation of Louisiana black bears (*Ursus americanus luteolus*) as detected through non-invasive sampling. Conservation Genetics 5, 647-659.

Truvé, J. 2004: Pigs in space. Movement, dispersal and geographic expansion of wild boar (*Sus scrofa*) in Sweden. Dissertation, University of Göteborg; ISBN 91-88376-25-7.

Valière, N. 2002: GIMLET: a computer program for analysing genetic individual identification data. Molecular Ecology Notes 2, 377-379.

Valiére, N., Bonenfant, C., Toigo, C., Luikart, G., Gaillard, J.-M., Klein, F. 2006: Importance of a pilot study for non-invasive genetic sampling: genotyping errors and population size estimation in red deer. Conservation Genetics 8, 69-78.

Vassant, J. 1996: Evolution of wild boar populations and damage to crops in France between 1978 and 1993. In: Bobek B, editor. 5th International Wild Boar and Suidae Symposium. Abstracts. Krakow, Poland

Vicente, J., Segalés, J., Höfle, U., Balasch, M., Plana-Duränm J., Domingo, M., Gortazàr, C. 2004: Epidemiological study on porcine circovirus type 2 (PCV2) infection in the European wild boar (*Sus scrofa*). Veterinary Research 35, 243-253.

Waits, L.P., Paetkau, D. 2005: Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. Journal of Wildlife Management 69, 1419-1433.

Waits, L.P., Luikart, G., Taberlet, P. 2001: Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10, 249-256.
Ward, A.I. 2005: Expanding ranges of wild and feral deer in Great Britain. Mammal Review 35, 165-173.

Wasser, S.K., Davenport, B., Ramage, E.R., Hunt, K.E., Parker, M., Clarke, C., Stenhouse, G. 2004: Faeces detection dogs in wildlife research and management: application to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. Canadian Journal of Zoology 82, 475-492.

Weiß, A. 1993 Pflege- und Entwicklungsplan Naturpark Pfälzerwald. Verein Naturpark Pfälzerwald e.V., Bad Dürkheim [in German].

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., Andersen, D.R., Burnham, K.P., Otis, D.L. 1982: Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory Report LA-8787-NERP, Los Alamos, New Mexico, 235 pp.

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

Wilberg, M.J., Dreher, B.P. 2004: GENECAP: a program for analysis of multilocus genotype data for non-invasive sampling and capture-recapture population estimation. Molecular Ecology Notes 4, 783-785.

Wilson, K.R., Anderson, D.R. 1985: Evaluation of two density estimators of small mammal population size. Journal of Mammalogy 66, 31-21.

Williams, B.K., Nichols, J.D., Conroy, M.J. 2002: Analysis and management of animal populations. Academic Press, San Diego, California, USA, 817 pp.

Wilson, G.J., Frantz, A,C, Pope, L.C., Roper, T.J., Burke, T.A., Cheeseman, C.L., Delahay, R.J. 2003: Estimation of badger abundance using faecal DNA typing. Journal of Applied Ecology 40, 658-666.

Woods, J.G., Paetkau, D., Lewis, D., McLellan, B.N., Proctor, M., Strobeck, C. 1999: Genetic tagging of free-ranging black and brown bears. Wildlife Society Bulletin 27, 616-627.

Zhan, X., Li, M., Zhang, Z., Goosens, B., Chen, Y., Wang, H., Bruford, M.W., Wei, F. 2006: Molecular censusing doubles giant panda population estimate in a key nature reserve. Current Biology 16, 451-452.