# Can hair traps sample wild boar () randomly for the purpose of non-invasive population estimation?

Cornelia Ebert, Ditmar Huckschlag, Holger K. Schulz, Ulf Hohmann

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ORIGINAL PAPER

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

**Cornelia Ebert · Ditmar Huckschlag · Holger K. Schulz · Ulf Hohmann**

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**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 2,124 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 (Sáez-Royuela and 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 boar into urban areas cause ecological and epidemiological concerns (Maillard et al. 1996; Vassant 1996; Schnidig-Petrig and 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).

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C. Ebert
Department Wildlife Ecology and Wildlife Management,
University of Freiburg,
Tennenbacher Strasse 4,
79106 Freiburg, Germany

C. Ebert (✉) · D. Huckschlag · U. Hohmann
Department of Forest and Wildlife Ecology,
Research Institute for Forestry and Forest
Ecology of Rhineland-Palatinate,
Hauptstrasse 16,
67705 Trippstadt, Germany
e-mail: cebert@gmx.de

H. K. Schulz
Institute of Environmental Sciences,
University of Koblenz-Landau,
Fortstrasse 7,
76829 Landau, Germany

Traditional approaches to population estimation of wild boar and other ungulates include hunting bag analysis (Boitani et al. 1995; Acevedo et al. 2006), direct sightings (Groot Bruinderink and 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 and 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 and Burnham 2005; 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 and Mangel 1989; Bellemain et al. 2005; Petit and Valière 2005; Fickel and 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 4,000 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 600–1,000 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 km$^2$ (range, 1.14 to 5.23 individuals per km$^2$ and year; Reis 2006)

### 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 four 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

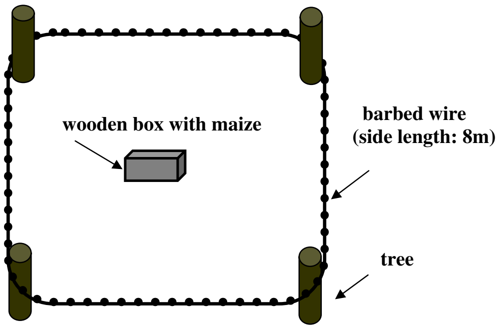



**Fig. 1** Scheme of a hair trap. The barbed wire is stretched in a height of 30–40 cm around four suitable trees with a total length of approximately 32 m. To get access to the bait, wild boar have to pass underneath the wire. The wire barbs serve as hair-snaring device

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 four 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 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.

**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 | 2,073 | 51 |

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).

## Results

Between 20.4.2006 and 10.8.2006, 216 visits of wild boar at two 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 morning (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.

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 (Fig. 3a and b). 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 d), even though the distribution of crossings was slightly less clumped compared to the 'centralized' baiting mode.

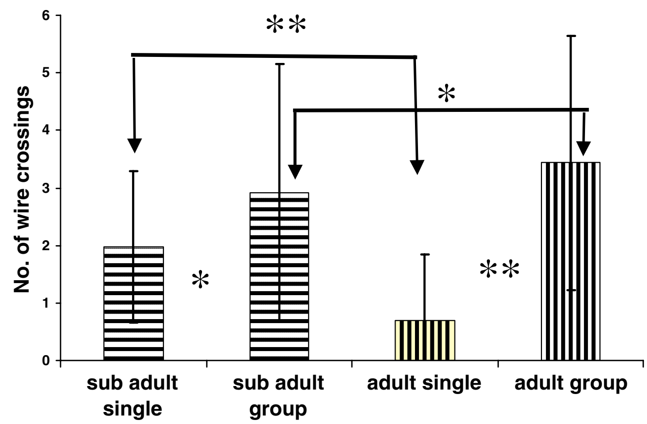
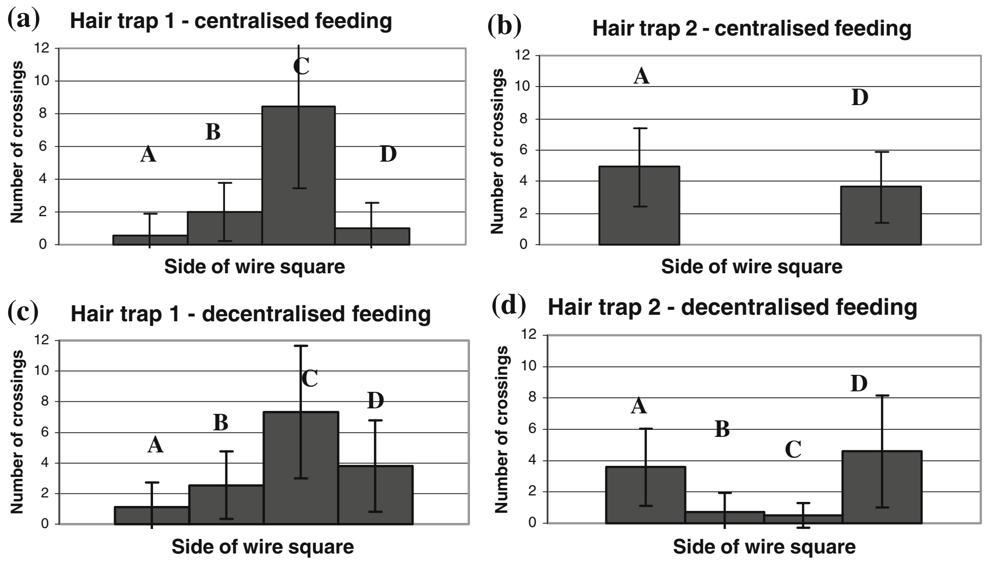



**Fig. 2** Wire crossing behaviour of adult and subadult wild boar as observed when visiting a hair trap. The number of crossings is shown for animals visiting hair traps as part of a group and for single visitors of each of the two observed age classes separately. Significant differences among and between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

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.

**Fig. 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




## 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 and 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 cannot 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 minimise 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 and 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 and 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 and Strobeck 2000; Mowat and 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 analyse 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 six 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 one 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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