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15 Abstract

Mammalian carnivore communities affect entire ecosystem functioning and structure. However, their large spatial requirements, preferred habitats, low densities, and elusive behavior deems them difficult to study. In recent years, non-invasive techniques have become much more common as they can be used to monitor multiple carnivore species across large areas at a relatively modest cost. Hair snares have the potential to fulfill such requirements, but have rarely been tested in Europe. Our objective was to quantitatively assess the effectiveness of hair snares for surveying mesocarnivores in the Iberian Peninsula (Southwestern Europe), by comparison with camera trapping. We used an occupancy modeling framework to assess method-specific detectability and occupancy estimates, and hypothesized that detection probabilities would be influenced by season, sampling method, and habitat related variables.

A total of 163 hair samples were collected, of which 136 potentially belonged to mesocarnivores. Genetic identification success varied with diagnostic method: 25.2% of identification success using mitochondrial CR, and 9.9% using the IRBP nuclear gene. Naïve occupancy estimates were, in average, 5.3 ± 1.2 times higher with camera trapping than with hair snaring, and method-specific detection probabilities revealed that camera traps were, in average, 6.7 ± 1.1 times more effective in detecting target species. Overall, few site-specific covariates revealed significant effects on mesocarnivore detectability.

Camera traps were a more efficient method for detecting mesocarnivores and estimating their occurrence when compared to hair snares. To improve our hair snares' low detection probabilities, we suggest increasing the number of sampling occasions and the frequency at which hair snares are checked. With some refinements to increase detection rates and the success of genetic identification, hair snaring methods may be valuable for providing deeper insights into population parameters, attained through adequate analysis of genetic information, that is not possible with camera traps.

38 Keywords: Noninvasive sampling, monitoring, molecular methods, occupancy, detection probability, carnivores

41 Introduction

Carnivores have cascading effects on entire ecosystems despite being relative sparse across landscapes (Gompper et al. 2006). As a result, carnivores are often the target of conservation efforts and an increasing number of studies have focused on assessing their density, relative abundance, or occupancy across large geographical areas (Gompper et al. 2006, Linkie et al. 2007). However, the challenges involved with monitoring carnivores are numerous. The majority of carnivores have large spatial requirements, often live in remote and rugged habitats, occur at low densities, and are nocturnal and elusive (Long et al. 2007, Mills 1996). Invasive techniques, such as mark-recapture or radiocollaring, are impractical to apply across large spatial scales since they are time-consuming, have high costs, and involve complex logistical requirements. Non-invasive techniques are therefore becoming much more common as they can be used to monitor multiple carnivore species across large areas at a relatively modest cost (Johnson et al. 2009, Weaver et al. 2005, Zielinski et al. 2006).

Camera traps and hair snares, two non-invasive techniques, are often used to confirm the presence of a species. Camera traps have successfully documented the presence of a vast array of common and rare mammals including felids, ursids, viverrids, mustelids, and cervids (Baldwin and Bender 2008, Johnson et al. 2009, Linkie et al. 2007, Tobler et al. 2009). Camera traps generally have high detection rates (Long et al. 2007, O'Connell et al. 2006) but only permit species identification if patterns in the pelage or specific markings allow individual identification. Hair snares, conversely, permit individual and sexual identification (using genetic methods) in addition to species identification, and recently have been extensively used to detect several mammal species (Kendall et al. 2009, Mills 1996, Ruell and Crooks 2007). The complementary individual identification provided by hair snares can be used to study the spatial structure, demography and occurrence of carnivore populations (Davoli et al. 2013; Zielinski et al. 2006).

62 The success of camera traps and hair snares at detecting animals varies across species and habitats. Thus, 63 quantifying the efficacy and potential biases of these techniques would help inform researchers and managers on 64 what sampling method(s) and survey design can be used to optimally achieve their research objectives (Nichols et 65 al. 2008). The ability to effectively and efficiently monitor carnivores is particularly critical in Southwestern (SW) Europe, since it has a diverse mammalian carnivore community, and where research studies and funding forconservation are limited in comparison to North America and other parts of Europe.

Using an occupancy modeling framework, we aimed to quantitatively assess the effectiveness of hair snares for surveying Iberian mesocarnivores, by investigating how sampling method (i.e., hair snares and camera trap surveys) affects the ability to detect and estimate species' occupancy. Occupancy modeling allows the estimation of method-specific detection probabilities, and consequently the sampling effort required to determine the occupancy status of each target species using camera traps vs. hair snares (Bailey et al. 2007). We hypothesized that site-specific covariates such as distance to water, habitat type, slope or elevation would influence target species behavior, and consequently, their detectability. Detection is also expected to be influenced by season and sampling method (O'Connell et al. 2006, Royle and Nichols 2003). Therefore, by controlling for these external factors potentially influencing detectability, we explored whether a hair snaring sampling protocol would provide adequate data for mesocarnivore population monitoring. As detection by rub stations is dependent on a behavioral response elicited by a lure or bait, we anticipated that detectability would be lower by hair snaring than by camera trapping.

79 Methods

80 Study areas

This study was performed in two different protected areas within the Mediterranean bioclimatic region of the Iberian Peninsula (Rivas-Martínez et al. 2004): the Guadiana Valley Natural Park (GVNP; Portugal; N 27º40'50'', W 7°44'30''), and the Cabañeros National Park (CNP; Spain; N 39°20'10'', W 4°25'50''). A study area of approximately 6000ha within each park was selected based on the criteria of ecosystem conservation status and logistic factors. The GVNP is located in the Guadiana River basin (Southeastern Portugal), the most important ecological corridor in southern Portugal, and harbors some of the most endangered species in Europe (ICN 2006, Sarmento et al. 2004). Small game hunting is a major economic driver within GNVP, and predator control directed towards red fox (Vulpes vulpes) and Egyptian mongoose (Herpestes ichneumon) is legally allowed. The landscape is highly fragmented with cereal croplands and agroforestry systems ('Montado') of stone pine Pinus pinea L. and holm oak Quercus ilex L. Scrubland patches are mainly associated with steeper slopes and elevation ridges (Costa et al. 1998, Monterroso et al. 2009). The CNP is located in the Castilla La-Mancha Spanish community, and is

dominated by *Pyro-Quercetum rotundifoliae* series and other sub-serial stages (Rivas-Martinez 1981), especially
associated with the steeper slopes, higher elevations and main water bodies. The landscape at the central lower part
of this study area constitutes a savannah-like system, with holm oak trees scattered within a grassland matrix
(García-Canseco 1997). Neither hunting activity nor predator control is allowed.

97 Survey methods and design

The sampling design was based on a sampling grid composed by 1-km² grid cells, which was superimposed over each study area. Sampling devices were deployed at grid cell vertexes, alternating between camera traps and hair snares. As a result, all cameras and all hair snares were approximately 1.4km apart, promoting method-specific independence. Study areas were surveyed in August-October 2009 (hereafter autumn season) and in February-April 2010 (hereafter spring season) for a period \geq 28 days, and assumed occupancy was constant during each survey period (MacKenzie et al. 2002). All procedures were performed in accordance with the guidelines for the care of mammals, as approved by the Portuguese Nature and Biodiversity Institute and the Animal Experimentation Ethic Committee of the University of Castilla La-Mancha (process nr. PP1104.3).

Hair snares on baited rub stations consisted of hair collection structures and scent lures (Kendall and Mckelvey 2008), and were set at 38 and 29 sampling locations in CNP and GVNP, respectively. Hair collection structures included both barbed rub pads and adhesive pads. This design exploits the cheek-rubbing behavior of felids, the neck-rubbing behavior of canids, and has been found to detect other mesocarnivores (e.g., mustelids) as by-catches (Kendall and Mckelvey 2008). Rub stations comprised a 50×5×5cm wooden stake, on which four 5x3cm pieces of dog wire (one at each side of the stake) were glued at 20 to 30cm above the ground. Below the dog wire, we covered the stake with sticky-side-out tape, which functioned as an adhesive pad. The attractants were deployed in separated, perforated plastic tubes supported by the wooden stake, at a distance of 10–15cm from each other (Monterroso et al. 2011). A volume of 5mL of each attractant was sprayed into a cotton gaze held inside each plastic tube. The selected attractants were Lynx urine and Valerian, which have been described as efficient in attracting mesocarnivores (Monterroso et al. 2011, Steyer et al. 2013). Hair snares were monitored and scent lures replenished every 7 days. We collected hairs with tweezers, stored them in plastic vials with ethanol (96%) and then kept at room temperature until lab processing. Hair samples were identified under a microscope by analyzing its medular and cuticular

structure with the aid of specific guides (e.g. Teerink 1991). Hair was identified as either under hair (UH), type 1 (GH1) or type 2 (GH2) guard hair. GH1 hair is usually stiff and firm, and occurs very often within pelage. It can be slightly wavy or bent. In GH2 hair the shaft is usually straight and forms an angle with the shield (Debelica and Thies 2009). Subsequently, samples were identified by molecular methods. Species assignment was performed using two diagnostic methods described by Oliveira et al. (2010; interphotoreceptor retinoid-binding protein, IRBP, fragment) and Palomares et al. (2002; domain 1 of the mitochondrial control region. CR), following the procedures described by Monterroso et al. (2012). Aligned IRBP and CR sequences were compared with the corresponding regions from the target species available in the GenBank and in CIBIO's genetic database. Both markers were consistently used to increase identification confidence. Whenever hair samples, collected from the same hair snare in the same sampling occasion, were identified as belonging to the same species from their medular and cuticular structure, they were used together for DNA extraction and molecular identification. Otherwise, single hair samples were analyzed idependently.

Leaf River IR5 infrared-triggered digital cameras (LeafRiver OutDoor Products, Taylorsville, Mississippi, USA) were deployed at 38 and 32 sampling locations in CNP and GVNP, respectively. A circular area of 250-m radius surrounding each grid-cell vertex was inspected for carnivore paths prior to camera trap placement. The final location of camera traps corresponded to areas of easy access and potentially good detection probability within the mentioned buffer. Cameras were then mounted on trees approximately 0.5 - 1.0m off the ground and set to record time and date when triggered. We programmed cameras to fire a burst of three photos when triggered, and with the minimal delay time possible (< 1min).

In order to enable adequate comparisons between sampling methods, the same attractants used in hair snares were used to attract animals to camera traps. Therefore, the same structure built for hair snares (but without the dog wire and adhesive tape) was set at a distance of 2-3m of camera traps. Scent lures at camera stations were replenished in 7 days intervals, when stations were checked for batteries and to change memory cards.

143 Occupancy modeling

Likelihood-based occupancy modeling was used to estimate detection probability (*P*), given presence, and the
probability of occupancy (*D*; MacKenzie et al. 2002, Mackenzie et al. 2006). To account for potential

heterogeneity in probabilities of occupancy and detection, and to evaluate our a priori hypotheses we assessed four site-specific covariates at the local scale: elevation, slope, distance to water and habitat type (forest, shrub or grassland). These covariates were assessed at each sampling location (camera trap or hair snare). We extracted elevation and slope data from the ASTER (Advanced Spaceborne Thermal Emission and Reflection radiometer) global digital elevation model (GDEM: www.gdem.aster.ersdac.or.jp), which has a spatial resolution of 30m; and estimated distance to water by measuring the linear distance from the sampling site to the nearest water source (i.e., river, lake, or reservoir). Habitat type was reclassified into three major structural types: forest, shrub and grassland cover from vegetation geographic information system coverages of CNP and GVNP, with a spatial resolution of 30m, and was assigned to each sampling site (camera trap or hair snare) according to its exact location.

We divided survey periods into four 1-week sampling occasions during which the detection/non-detection data on each target species was recorded. We created species-specific detection histories, allowing us to assess factors that may affect species-specific detection. The probabilities of detecting target species given they occupy a site (i.e. *P*) were estimated from their detection histories. Missing values during a sampling occasion resulted from cameras malfunctioning or temporary inability to access a camera trap or hair snare.

Multi-season occupancy models were developed in PRESENCE 5.8 (Hines and Mackenzie 2013) to estimate species and method-specific occupancy and detection probabilities. A set of candidate models was built for each speciesstudy area combination based on our *a priori* hypotheses. We modeled occupancy as constant across all sampling sites and constant *vs.* dependent on sampling season. Detection probability was modeled as constant or dependent on season, sampling occasion or site-covariates.

As we wanted to assess the effect of detection method (i.e. hair snare vs. camera trap) on detection probabilities we tested the simplest models with and without a detection method covariate: models $\psi(.)p(.),\psi(.)p(method),\psi(season)p(.),\psi(.)$ $\psi(\text{season})p(\text{method})$. If the effect of method was found to be significant, we developed the models further, constraining them to always include the method covariate. We used Spearman's rank correlation (r_s) to test for collinearity among the landscape variables; if variables were correlated ($r_s > 0.70$) we kept the variable with the greatest univariate effect size (β /SE) as a potential covariate for the probability of detection (Zar 2005). We estimated overall AIC weights for individual variables by summing the AIC weights of all the candidate models in which they were included (Mackenzie et al. 2006). If no single model accounted for > 90% of the total model

weights, we model-averaged by extracting the top 95% model confidence set and recalculating model weights
(Burnham and Anderson 2002). Model averaged estimates were calculated using the spreadsheet developed by B.
Mitchell (<u>http://www.uvm.edu/%7Ebmitchel/software.html</u>).

Finally, we estimated the number of hair snare surveys and the number of camera trap surveys, n_i , required to achieve a specified probability of detection. We estimated n_i following Long et al. (2007): $P = 1 - (1 - p_i)^{n_i}$. The effectiveness of camera traps and hair snares for mesocarnivores using 3 indicators: (1) naïve occupancy estimates (i.e. proportion of sites where the target species was detected by a single sampling method in a single season), (2) method-specific estimates of the probabilities of occupancy and detection; and (3) number of surveys required using each method to reach a designated detection probability.

182 Results

A total of 163 hair samples were collected in hair snare stations (Table 1). CNP accounted with 43 and 70 samples in autumn and spring seasons, respectively, while 24 and 26 samples were obtained from the same seasons at GVNP. The average number of hairs collected per sample was 5.42 ± 0.35 (mean \pm SE). Hair samples that were unequivocally identified by their microscopic structure as belonging to non-target species (e.g. ungulates or lagomorphs) were not sent for genetic analysis (n=27). However, potential carnivores' or unidentified hair samples were sent for genetic analysis, and consisted of 83.4% of the total samples (n=136).

The genetic identification success varied with diagnostic method: 25.2% of identification success using
mitochondrial CR, and 9.9% using the IRBP nuclear gene.

Hair samples were identified as belonging to red fox, stone marten, and European wildcat when employing conventional microscopic methods; no samples were identified as belonging to common genets, European badger, or Egyptian mongoose. However, employing genetic methods hair samples were identified as belonging to red fox, genet, and stone martens; no samples were identified as belonging to European wildcat, European badger, or Egyptian mongoose. 25 samples from CNP were genetically identified as red fox: 15 from autumn and 10 from spring seasons; 5 samples from GVNP were red fox: 2 from autumn and 3 from spring seasons. Genetically identified genet hair was only obtained at CNP, with one sample from each season. Only one hair sample collected at GVNP during the spring season was genetically confirmed as stone marten.

From all of the genetically confirmed red fox hair samples (n=30), 67% contained under hair (UH) while 50% and 10% contained GH2 and GH1 guard hair, respectively. Seventy-three percent of the hair samples were collected from dog wire brush and 27% from adhesive tape. Genetically confirmed common genet samples (n=2), were either UH (n=1) or GH1 (n=1). Both genet hair samples were collected from dog wire brush. The only genetically confirmed stone marten hair sample consisted of GH2 guard hair, and it was obtained from the adhesive tape.

With camera trapping methods we were able to detect red foxes, European wildcats, common genets, stone martens,
Egyptian mongooses and Eurasian badgers at GVNP in both seasons (Table 2). At CNP, we were able to detect the
same species during autumn using camera traps. However, the Egyptian mongoose was not detected during autumn.
Although mesocarnivore species composition was similar between the two study areas, their spatial distribution
differed, as supported by their naïve occupancy estimates (Table 2).

209 Naïve estimates, occupancy and detection probabilities

We had a greater number of detections via camera trapping than we did via hair snares. When both methods detected the target species, naïve occupancy estimates were, on average, $5.3 (\pm 1.2)$ times higher with camera trapping than with hair snaring (table 2). For the species undetected by hair snares, naïve occupancy based on camera traps were always < 10% in CNP (Table 2). Conversely, at GVNP species undetected by hair snaring displayed naïve occupancy estimates ranging from 3 to 23% (Table 2).

The limited numbers of detections prevented us from modeling common genet at GVNP and European wildcat,
Eurasian badger, and Egyptian mongoose in both study areas. For the species that did have sufficient numbers of
detections, our estimated probabilities of occupancy were, on average, 31.5% (± 3.7%) greater than our overall naïve
estimates (Tables 2 and 3).

Method-specific detection probabilities revealed that camera traps were, on average, $6.7 (\pm 1.1)$ times more effective in detecting target species than hair snares (Table 3). Given presence, red foxes had, on average, a 49.9% (± 10.4%) and 14.2% (± 5.4%) chance of being detected by camera traps and hair snares, respectively, in a give sampling occasion (Table 3). The mean probability of detecting stone martens by camera trapping was 21.7% (± 3.2%) and $3.5\% (\pm 0.6\%)$ by camera trapping and hair snaring, respectively (Table 3). Common genets at CNP had mean chance of being detected of 20.1% (± 1.2%) by camera trapping and 2.1% (± 0.2%) by hair snaring (Table 3).

The top ranked models for red fox consistently included habitat type at CNP and elevation at GVNP. Distance to water was included in three, and slope in one of the top ranked models at CNP; whilst slope, elevation and distance to water were each included at a single model of the top ranked models at GVNP. The top ranked models for common genet at CNP consistently included distance to water, but elevation also appeared in 5 of these models. Slope was included in two of these models and habitat type in one.

The effect of detection method was positive and significant across species and study areas, with $\hat{\beta}$ estimates ranging from 1.75 to 2.56 (Table 4). The 95% confidence intervals of all red fox model-averaged covariates overlapped 0.0 at GVNP. However, a significant seasonal influence was detected at CNP, with the probability of detecting a red fox being significantly higher in spring than in autumn (Table 4). Elevation also showed a significant negative effect on detection probability at CNP (table 4). For stone martens at GVNP, season was the only covariate to significantly influence detectability with P decreasing from autumn to spring. At CNP, there were no observable covariate effects (Table 4). For genets, distance to water significantly negatively influenced detection probability (Table 4). All remaining variables' coefficients exhibited 95% confidence intervals that overlapped 0.0 (Table 4).

A greater number of 1-week sampling occasions are required to attain a given detection probability when employing hair snares than when employing camera traps (Figure 1). Based on the obtained detection probabilities, camera traps would have to be deployed, on average, for ≥ 4 1-week sampling occasions to confirm red fox occupancy, with 95% accuracy. In order to achieve the same level of accuracy, ≥ 20 1-week occasions are required when employing hair snares. Additionally, ≥ 12 and 13 camera trapping sampling occasions are required to confirm stone marten and genet occupancy, respectively, with 95% accuracy (Figure 1). It would take 6.9 and 10.8 times longer to achieve the same confidence level for stone martens and genets, respectively, if using hair snares.

245 Discussion

Camera traps were a more efficient method for detecting mesocarnivores and estimating their occurrence when
compared to hair snares. These results are consistent with previous studies done in North America (Comer et al.
2011, Long et al. 2007, O'Connell et al. 2006). We detected a total of six mesocarnivore species in each of the
study areas when employing camera trapping, in comparison to only three mesocarnivore species in each of the
study areas when employing hair snares. When both methods were able to detect a target species, partial naïve (raw)

occupancy estimates were 7.7 ± 1.9% higher when assessed through camera trapping than through hair snaring
methods. Lastly, we found that hair snares required a greater number of sampling occasions to attain a given
detection probability than camera traps. This suggests that our four-week sampling period would not have provided
adequate estimates of species occupancy in our study areas had we only employed hair snares.

A limited number of hairs were collected from hair snares (< 10 hairs/sample) and this number was reduced even further when considering the tufts of hair that yielded sufficient DNA for species identification. Our overall success of the molecular methods was rather low when compared to similar studies, which usually ranges from 40 to 80% (Weaver et al. 2005, Long et al. 2007, Stever et al. 2012). Three main factors may be responsible for our low success rates in genetic identification: low DNA quantity, low DNA quality and contamination (Kendall and Mckelvey 2008). Most hair collected from rub stations, such the ones used in our study, consists of shed hair. Shed hair can provide enough DNA for genetic species assignment if mitochondrial DNA is used (Mills et al. 2000, Riddle et al. 2003). However, the DNA quantity obtained of plucked hair is usually higher because it often contains follicles, which are the main source of DNA for analysis (Goossens et al. 1998). DNA quality can also be affected by exposure to harsh environmental conditions, especially environmental temperature (Nsubuga et al. 2004, Santini et al. 2007). Both of our study areas are located in the Mediterranean Bioclimatic region of the Iberian Peninsula, where ambient temperature often rises above 35°C during the warmer seasons (Hijmans et al. 2005, Rivas-Martínez et al. 2004). These warm temperatures could have decreased DNA quality in the autumn period. Further, the spring season corresponded to a period of heavy precipitation, which could have led to sample "wash", and a consequent reduction of DNA quality. Cross-contamination from multiple visits to the same station within a sampling occasion, can also reduce DNA identification success because mixed samples could lead to more multiple alleles at one or more diagnostic loci, preventing adequate genotyping (Mowat and Paetkau 2002). Reducing the time between station revisits could increase genetic identification success by preventing excessive exposure of hair DNA to environmental conditions and reducing the probability of multiple visits. However, a likely drawback of reducing the length of sampling occasions would be a reduction in detection probabilities and increase in survey costs (Long et al. 2007, Mowat and Paetkau 2002). Our sampling occasion length, 7 days, is similar to that used in other studies (e.g. Long et al. 2007, Stricker et al. 2012, Burki et al. 2010).

The baited hair snare model we tested (sensu Kendall and McKelvey 2008) required an active response from the target species in order to produce a detection (i.e. the rubbing behavior exhibited by most felid and canid species). Similar rub stations have been tested worldwide on a variety of species and yielded contrasting results. Long et al. (2007) failed to detect bobcats (Lynx rufus) in Vermont, USA, with rub pad hair snares, but successfully detected them with scat detection dogs and camera traps. However, they successfully detected black bears with all three methods. Comer et al. (2011) obtained low bobcat detection rates in Texas, USA, when compared to those obtained by camera traps. Using similar rub pads, Downey et al. (2007) failed to detect margays (Leopardus wiedii) at El Cielo Biosphere Reserve (Mexico), but obtained a 20.8% success in detecting gray foxes (Urocyon cinereoargenteus), whereas Castro-Arellano et al. (2008) were successful in detecting 67% of the medium and large mammals species known to be present. Steyer et al. (2012) were successful in identifying individual European wildcats with rub pad hair snares at a low-density area, in the Kellerwald-Edersee National Park, Germany. Even though cubby-like designs have been preferred for collecting hair from mustelids (Kendall and Mckelvey 2008), pine martens have been successfully detected by their hair using lure sticks at the Jura Mountains, Switzerland (Burki et al. 2010).

We used lynx urine and valerian extract solution as our scent lures because they have been found to elicit rubbing behavior in captive red foxes, European wildcats, common genets and Eurasian (Monterroso et al. 2011). We were surprised by the small number of wildcat hair samples collected in our study, especially in GVNP where a stable wildcat population is known to occur (Monterroso et al. 2009). Similar studies (with regard to hair collection structures and attractants) have proved effective for wildcat detection (Steyer et al. 2013) and estimation of population parameters (Kéry et al. 2011,). However, some studies have found valerian to be ineffective in attracting wildcats (Kilshaw & Macdonald, 2011; Anile et al. 2012), suggesting that genetic characteristics of wildcat populations could be related to their attractiveness towards valerian lure. Further field tests could help clarify the reasons for the poor performance of hair snares for detecting wildcats in our study areas.

Overall, a limited number of site-specific covariates revealed influence on the detectability of mesocarnivores. In
 CNP, we found the probability a red fox was detected was negatively related to elevation and the probability a genet
 was detected was negatively related to distance to water. We suggest that this is because the foxes' scavenging
 behavior at CNP is related to the abundance of Red deer (*Cervus elaphus*) and Wild boar (*Sus scrofa*) carcasses at

lower elevations (García-Canseco 1997) and waterways provide abundant cover, food, and often serve as travel corridors (Rondinini and Boitani 2002, Santos et al. 2008). Given the close relationship between abundance and detectability (McCarthy et al. 2012), we would foxes were more abundant at lower elevations and genets closer to water.. In CNP, red fox were also more likely to be detected in autumn than in spring and in GVNP, stone marten were more likely to be detected in spring than in autumn. This was most likely the result of seasonal differences in the annual biological cycle of the target species. For example, the yearlings of most mesocarnivores disperse and incorporate the 'active' population in autumn. Thus, territoriality is more relaxed when compared to the spring season, which coincides with the breeding season of most species (Blanco 1998).

To our knowledge, this is the first study that evaluates the efficiency of hair snares for monitoring a mesocarnivore community in Europe. If individuals only need to be identified to the species-level, then our results suggest that camera trapping is a more efficient sampling method than hair snares. Other noninvasive methods, such as detection dogs or scat surveys, may also provide detection rates comparable to those of camera traps (Gompper et al. 2006, Long et al. 2007, O'Connell et al. 2006). However, because hair samples can be identified to the individual level through microsatellite analysis of nuclear DNA (Beja-Pereira et al. 2009), they allow for the estimation of population parameters such as density (Kéry et al. 2011), spatial organization (Davoli et al. 2012) or genetic diversity (Mullins et al. 2009).

Protected area administrations require adequate information on the status of wildlife populations through constant monitoring in order to detect population trends or sudden changes, and adjust management actions accordingly (Moriarty et al. 2011). Occupancy modeling, in combination with camera trap surveys, may be an ideal method for large-scale, long-term monitoring of wildlife populations as it provides information on the spatial distribution of species and patch-specific rates of colonization and extinction (MacKenzie et al. 2003, Moriarty et al. 2011). If management objectives, however, require deeper insights into population dynamics that can only be attained through analysis of genetic information (Kendall and Mckelvey 2008), then hair snaring may need to be employed. To improve the efficacy of hair snaring, we suggest increasing the number of sampling occasions (Bailey et al. 2007, O'Connell et al. 2006) and the frequency at which hair snares are checked. This will likely improve detection rates, minimize environmental degradation of DNA, and decrease incidence of cross-contamination. Additionally, depending on the target species, employing multiple types of hair snares (e.g., rub pads and cubby boxes) and

multiple types of lures at each station may increase the number of species detected and overall detection rates. We suggest that future studies test different hair snare protocols and sampling designs, perhaps through simulation studies, to increase the efficiency of hair snare techniques; namely, determining the optimal duration of sampling occasions and the design of snares that increases both detection probabilities and the success of molecular methods. Acknowledgments This work was partially supported by a PhD grant from the Fundac ão para a Cie ncia e a Tecnologia (FCT) to PM (SFRH/BD/37795/2007) and two research projects, one from the Spanish National Plan (project ref: CGL2009-10741) funded by the Spanish Ministry of Science and Innovation and EU-FEDER funds, and one from the Spanish Organismo Autónomo Parques Nacionales (project ref: OAPN 28 341 352/2011). We thank Pedro Moreira, Ricardo Silva, Rafaela Carreira and Francisco Díaz-Ruiz for their assistance 30 342 during the fieldwork. We acknowledge the staff from Cabañeros National Park, especially Angel Gómez, the staff from Vale do Guadiana Natural Park for their support during data collection. References Anile S, Arrabito C, Mazzamuto MV, Scornavacca D, Ragni B (2012) A non-invasive monitoring on European wildcat (Felis silvestris silvestris Schreber, 1777) in Sicily using hair trapping and camera trapping: does scented lure work? Hystrix, the It J Mamm 23:44-49. Doi: 10.1007/s10344-012-0644-0 Bailey L, Hines JE, Nichols JD, MacKenzie D (2007) Sampling design trade-offs in occupancy studies with imperfect detection: examples and software. Ecol Appl 17:281-90. Doi: 10.1890/1051-0761(2007)017[0281:SDTIOS]2.0.CO;2 Baldwin RA, Bender LC (2008) Distribution, Occupancy, and Habitat Correlates of American Martens (Martes americana) in Rocky Mountain National Park, Colorado. J Mammal 89:419-427. Doi: 10.1644/07-MAMM-A-053R1.1 Beja-Pereira A, Oliveira R, Alves PC, Schwartz MK, Luikart G (2009) Advancing ecological understandings through technological transformations in noninvasive genetics. Mol Ecol Resour 9:1279–301. Doi: 10.1111/j.1755-0998.2009.02699.x Blanco JC (1998) Mamíferos de España. Planeta, Barcelona,.

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29 30 31	485	
32 33 34	486	Figures:
34 35 36 37	487 488	Figure 1. Mean estimated sampling occasions (weeks) required to attain a given detection probability, given species presence, for a) red foxes, b) stone martens, and c) common genets.
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		Autumn	Spring	Autumn	Spring	Total
Number of samples collected		43	70	24	26	163
Presence of intact hair		0.63 (27)	0.90 (63)	0.38 (9)	0.88 (23)	0.75 (122)
	UH	0.42 (18)	0.56 (39)	0.54(13)	0.58 (15)	0.52 (85)
Hair type	GH	0.60 (26)	0.39 (27)	0.46(11)	0.27 (7)	0.44 (71)
1	GH2	0.28 (12)	0.07 (5)	0.00 (0)	0.08 (2)	0.12 (19)
• • •	Brush	0.86 (37)	0.81 (57)	1.00 (24)	0.85 (22)	0.86 (140
Collection device	Tape	0.14(6)	0.21 (15)	0.00 (0)	0.15 (4)	0.15(25)
Samples sent for genetic ID		0.77 (33)	0.96 (67)	0.50 (12)	0.92 (24)	0.83 (136
	Amplification	0.85 (28)	0.27 (18)	0.25 (3)	0.38 (9)	0.43 (58)
CR (mitochondrial)	Sequencing	0.85 (28)	0.21 (14)	0.25 (3)	0.38 (9)	0.40 (54)
	Identification	0.52 (17)	0.13 (9)	0.17 (2)	0.25 (6)	0.25 (34)
	Amplification	0.36 (12)	0.16 (11)	0.17 (2)	0.50 (12)	0.27 (37)
IRBP (nuclear)	Sequencing	0.27 (9)	0.10(7)	0.08 (1)	0.50 (12)	0.21 (29)
	Identification	0.24 (8)	0.06(4)	0.00(0)	0.00(0)	0.09 (12)

ortion nositive (number of samples) Table 1. Proportion of samples obtained of each hair type, collection structure, and results from molecular analysis obtained from hair snaring methods at Condision Mathematical Ports (CMM) in the outputs 2000 and analysis of 2010 Descention acciding (analysis of source) f 2010 Dr. Dark (CND) in the Natio d Cabaña (GVND) Table 2. Naïve occupancy estimates (# sites with detections/# sampling sites) of mesocarnivores based on camera-trapping and hair snaring at Guadiana Valley Natural Park (GVNP) and Cabañeros National Park (CNP) in the autumn 2009 and spring of 2010.

		Overall naïv	e estimates		Partial naï	ve estimates	
Study area	Species	A 110111	Contine	Autu	um	Spr	ing
		Automatic	gmude	Camera traps	Hair snares	Camera traps	Hair snare
	Red fox	0.23	0.20	0.41	0.03	0.25	0.14
	Stone marten	0.16	0.36	0.25	0.07	0.63	0.14
	Common genet	0.08	0.12	0.16	0.00	0.22	0.00
	European wildcat	0.26	0.13	0.44	0.07	0.25	0.00
	Eurasian badger	0.03	0.07	0.06	0.00	0.13	0.00
	Egyptian mongoose	0.11	0.18	0.22	0.00	0.34	0.00
	Red fox	0.56	0.65	06.0	0.42	0.88	0.03
	Stone marten	0.26	0.22	0.46	0.05	0.28	0.16
	Common genet	0.14	0.12	0.27	0.03	0.20	0.05
CINE	European wildcat	0.04	0.08	0.07	0.00	0.15	0.00
	Eurasian badger	0.06	0.04	0.12	0.00	0.08	0.00
	Egyptian mongoose	0.00	0.03	0.00	0.00	0.05	0.00

Valley Natur	ral Park (GVNP)	and Cabañeros National	Park (CNP), in aut	umn 2009 and spring 2	10000 Estimates \pm SE.	r-uapping and nait sual	lig al Ouaulalia
Cturder outon	Douomotou	Red fox		Stone mai	ten	Common gen	et
oluuy area	r ar anneuer	Autumn	Spring	Autumn	Spring	Autumn	Spring

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 0.02 ± 0.02 0.21 ± 0.11

 0.20 ± 0.09 0.04 ± 0.02

 0.04 ± 0.02

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 0.67 ± 0.21

 0.64 ± 0.22

 0.31 ± 0.08 0.05 ± 0.03

 0.16 ± 0.09 0.02 ± 0.02

 0.32 ± 0.14 0.06 ± 0.04 0.79 ± 0.15 0.74 ± 0.15 0.29 ± 0.14

 0.44 ± 0.17

 0.44 ± 0.15 0.34 ± 0.13 0.06 ± 0.04 0.81 ± 0.15 0.60 ± 0.18 0.17 ± 0.07

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 $P_{hairsnares}$

 $P_{cameras}$

GVNP

 $P_{hairsnares}$

 $P_{cameras}$ Ŷ

CNP

 0.70 ± 0.19

 0.71 ± 0.15

nev $(i\hat{h})$ and method-enerific detection probabilities (P) of red foxes based on camera-tranning and hair snaring at Guadiana Table 3 Model averaged occu

0000 - P - 70		Red fox	2	Stone marten		Comn	non genet
Study area	Covariate	AIC wgt	β [95% CI]	AIC wgt	$\hat{oldsymbol{eta}}$ [95% CI]	AIC wgt	$\hat{\beta}$ [95% CI]
	Intercept	1	-2.97 [-4.71; -1.24]	1	-4.42 [-6-16; -2.69]		ı
	Season	0.34	-1.09 [-4.27; 2.10]	0.32	-3.01 [-5.16; -0.87]	·	ı
	Method	1.00*	2.17 [0.96; 3.38]	1.00*	2.56 [1.48; 3.64]		ı
	Habitat: forest	0.75	0.39 [-0.64; 1.43]	0.75	-0.04 [-0.54; 0.45]	·	ı
GVNP	Habitat: shrub	0.75	1.38 [-0.65; 3.41]	0.75	-0.04 [-0.61; 0.54]	·	ı
	Distance to water	0.23	0.06 [-1.14; 1.25]	0.20	-0.27 [-1.79; 1.25]	ı	ı
	Elevation	0.27	-0.56 [-6.38; 5.26]	0.18	-1.64 [-8.14; 4.86]	ı	·
	Slope	0.20	-0.02 [-0.14; 0.10]	0.25	0.10 [-0.05; 0.25]	ı	ı
	Intercept	T	3.03 [-0.48; 6.53]	1	-4.52 [-8.88; -0.17]	-	-5.92 [-12.22; 0.3
	Season	0.98	3.71 [0.13; 7.28]	0.27	-1.15 [-5.60; 3.31]	0.35	-2.11 [-8.83; 4.6]
	Method	1.00*	2.17 [1.23; 3.10]	0.99*	1.75 [0.70; 2.80]	1.00*	2.51 [0.90; 4.12]
	Habitat: forest	0.23	-0.17 [-1.15; 0.80]	0.18	0.07 [-0.59; 0.74]	0.29	0.02 [-1.25; 1.29
CNF	Habitat: shrub	0.23	-0.07 [-0.53; 0.40]	0.18	0.05 [-0.38; 0.48]	0.29	0.23 [-0.83; 1.29
	Distance to water	0.60	-0.64 [-2.08: 0.81]	0.87	-1.98 [-4.57; 0.62]	0.93	-4.00 [-7.92; -0.0
	Elevation	0.95	-6.42 [-11.82; -1.03]	0.46	2.31 [-3.45; 8.07]	0.62	3.83 [-4.36; 12.03
	Slope	0.38	0.01 [-0.05; 0.07]	0.56	0.02 [-0.05: 0.09]	0.33	0.02 [-0.08; 0.11

Table 4. Model averaged variable weights and beta estimates ($\hat{\beta}$), with 95% confidence intervals, on detection probability (P) at Guadiana Valley Natural Park



