2. METHODS

2.1 Creating the system

Detecting changes in abundance on endangered species is a key issue for their conservation and for a basis of decision making in wildlife management. Because of their relatively low population sizes and elusive nature, carnivores are notoriously difficult to survey. Fortunately, sophisticated non-invasive methodologies for furthering our knowledge of carnivores are increasingly available. The cost-effective development of biochemical techniques, such as DNA analyses, permits accurate identification of an individual animal from remains such as scats and shed hairs. Camera-trap photography, track plates, scent stations, hair snagging, and genetic analyses can now be used in conjunction with traditional techniques -- such as snow tracking, trapping, and radio-telemetry -- to monitor carnivores. Because no single survey method is suitable for all species, we are using a suite of non-invasive techniques, found to be effective in Iberian lynx (Guzmán *et al.*, 2002) and other feline species. One important issue when sampling such a rare species as the Iberian lynx is using a method with high reliability that could detect, with substantial precision, presence and declines in population size. So, we decided not to use sighting reports as proofs of lynx existence since this method tends to give a fictional scenario of lynx numbers (Guzmán *et al.*, 2002). Personal interviews and questionnaires most times including misidentification of lynx, considerable errors on the sighting date and place.

For conducting this survey, we choose a combination of methods with high reliability and that could constitute a suitable data base for future lynx conservation. These methods were:

1- Sign searching;
2- Camera trapping;
3- Box trapping.

2.2 Sign searching techniques

The sign searching methodology was conducted using a geographic framework of 10 x 10 km UTM grid squares, defined upon lynx historical range estimated by previous studies (Palma, 1980; Ceia *et al.*, 1998) (Figure 3). For the survey we also incorporated several areas of lynx potential habitat outside its previously defined range (Figure 3). The field work consisted of a search for signs of lynx and rabbit presence and was undertaken between January 2002 and May 2003.

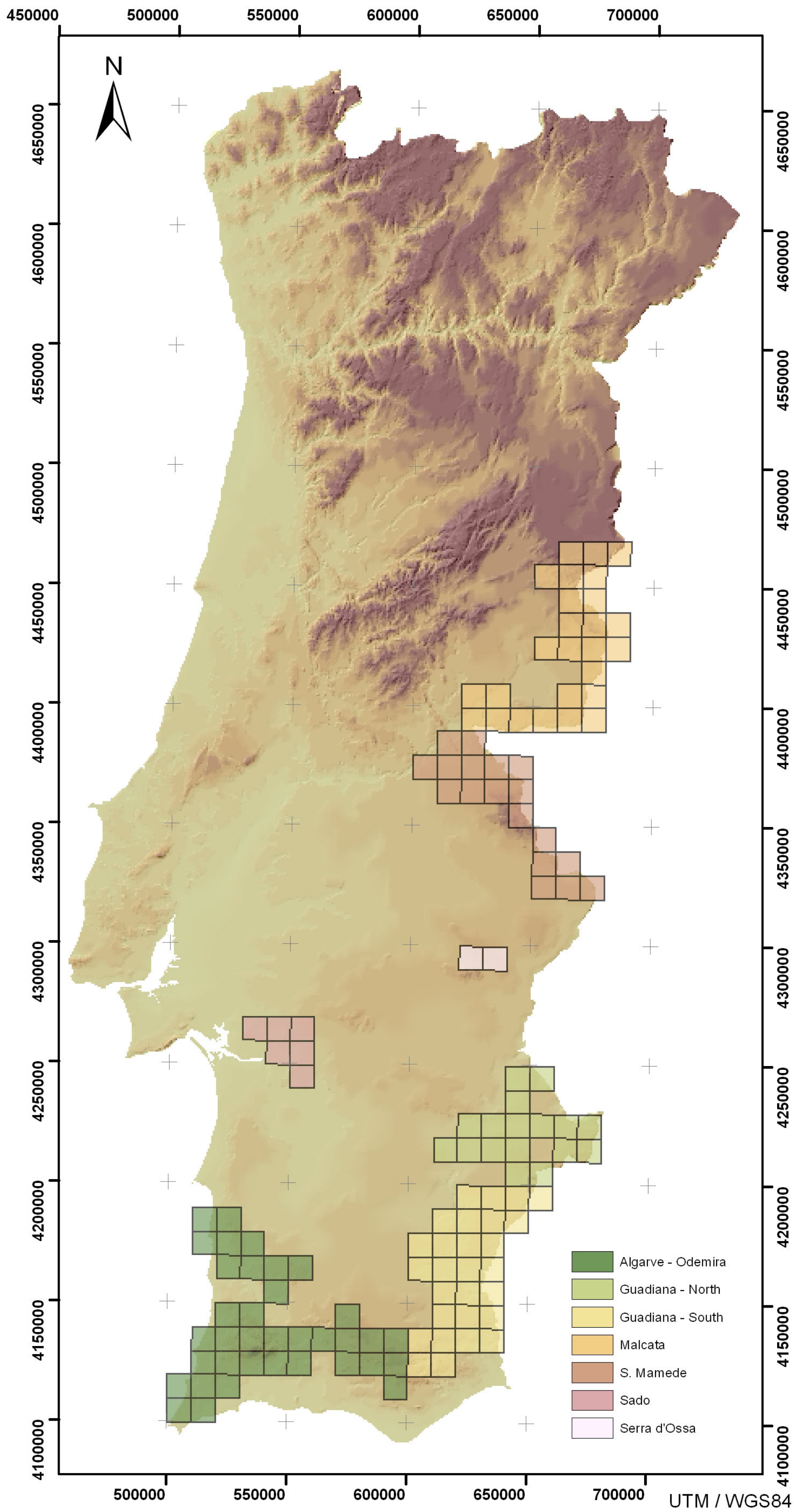

Figure 3 – UTM squares (10 x 10 km) surveyed for lynx presence during the current study.

2.2.1 Focus zones

In order to augment the probability of obtaining positive results and to obtain a more coarse-grain survey, we defined focus zones inside the UTM squares (Photograph 1). Focus zones are areas of particular interest where intensive effort was conducted; they are irregular in shape and they present natural characteristics suitable for lynx, namely:

1- Presence of Mediterranean scrubland patches with areas superior to 100 hectares;
2- Potential ecological corridors between suitable patches;
3- Medium or high rabbit density.

▲**Photograph 1** – Example of a focus zone on a sampled UTM square (10 x 10 km).

Focus zones were defined using the criteria of Palomares (2001) which describes an ideal habitat for lynx.

2.2.2 Collected data

Lynx

In focus zones searching efforts were conducted on roads, trails and rocky areas since the lynx uses mostly these spots for territorial marking. Mudded areas and snow covered territories (North range) were also searched for potential tracks.

When a potential sign was detected, its UTM geographic coordinates were recorded using a GPS navigator and deposition location was characterized by recording the following data, in a 20 m radium circle: 1) percentage of rocky cover; 2) percentage of grass cover; 3) percentage of shrub cover; 4) percentage of tree cover; 5) trees species. Percentage class were defined using the following scale:

0 (0%); 1 (1-10%); 2 (11-20%); 3 (21-40%); 4 (41-60%); 5 (61-80%); 6 (81-100%)

Lynx potential scats (Photograph 2)were collected using a protocol suitable for DNA analyses, in order to determinate their specific taxonomic origin (Palomares *et al.*, 2000; Pires & Fernandes, 2001).

▲**Photograph 2** – Potential lynx scat.

Recent advances in DNA techniques have opened the door for more accurate assessment of lynx distribution and so in the current project we gave emphasis to this methodology.
Scatological DNA analysis were performed in the following laboratories: Doñana Biological Station (Sevilla, Spain), Cáceres School of Veterinary (Cáceres, Spain), Laboratory of Genomic Diversity (Washington, USA).

Rabbit

Rabbit presence and density was obtained by counting latrines within the established transects on the focus zones. Latrines were divided into three groups according to the number of estimated pellets (Sarmento *et al.*, 2001): 1) type I – 1- 50 pellets; 2) type II – 51 to 125 pellets; 3) type III - > 125 pellets. Then, we use the index of rabbit abundance (RI) described in Sarmento *et al.* (2001), which is:

$$RI = \frac{12.510\ TIII + 5.10\ TII + TI}{CE}, \text{ being:}$$

TIII – number of type III latrines; TII – number of type II latrines; TI – number of type I latrines; CE – covered space.

Using the RI, we convert the data in rabbit abundance classes which were ranked using the following scale:

Class 0:RI = 0 - not detected
Class 1. RI from 1 to 10 - Low density
Class 2 . RI from 11 to 40: Low medium density
Class 3. RI from 41 to 70: High medium density
Class 4 . RI from 71 to 100: High density
Class 5. RI > 100: Very high density

Using the data obtained during the field work, conducted in Malcata since 1999 (Sarmento *et al*, 2003), we related the described scale with estimated rabbit densities, this procedure allowed us to establish density intervals for each class and potential implication upon lynx territorial behaviour (Palomares., 2001)(Table 2).

Table 2: Relation between rabbit abundance classes, estimated rabbit density and lynx social classes (from Sarmento *et al.*, 2001).

Class	RI	Estimated rabbit density (animals/he)	Estimated lynx response
0	0	0	Absent/vagrants
1	1-10	>0-0.75	Vagrants
2	11-40	0.75-2.0	Vagrants
3	41-70	2.0-4.5	Residents
4	71-100	4.5-10	Reproduction
5	>100	>10	Reproduction

2.2.3 Sampling effort

The sampling effort per square was defined according to the size of focus zones. Guzmán *et al.* (2002), on their status survey of Iberian lynx in Spain, established a minimum effort of 8 man/hour per square. For this work, we decided to calculate the sampling effort using previous field experiences. Using a GPS navigator, we determined the medium velocity of a field assistant and travelled distances in different size focus zones and, in order to obtain a suitable sampling, in different squares, we created a reference table (Table 3) that relates the percentage of suitable habitat with the minimum effort for a reliable sampling.

Table 3: Reference table to determine the minimum effort per square according to the percentage of suitable habitat.

% of potential habitat	Minimum effort (man/hour)	Estimated distance sampled
<10	4	17.2
11-20%	6	25.8
21-40	9	34.4
41-60	12	51.6
61-80	16	68.8
81-100	18	77.4

2.3 Camera trapping

The use of camera traps to detect elusive mammals, such as carnivores, has proved to be highly efficient (Cutler & Swann, 1999). The technique has the advantage of being cost-effective and providing positive species identification. Another advantage is the low perturbation effect in detecting cryptical animals with inconspicuous habits (Zielinsky *et al*, 1995). Currently, photographic trapping is successfully used in Spain, in the Iberian lynx national census and in other ecological studies (Guzman *et al.*, 2002), and is gaining popularity as a suitable method for this species detection. Recent results, obtained in Spain, attributed to this technique a substantial importance on determine lynx abundance and presence, since animals can be identified by natural features (e.g., pelage characteristics) which is an evidence that camera-trapping survey methodology is reliable for the purpose at hand.

2.3.1 Photographic devices

Two distinct camera devices were used: pressure plate triggering device (Photograph 3) and sensor heat activated CamTracker®(Photograph 4). In the first camera system the triggering device is as pressurised plate (dimensions of 30 × 30 cm) which is connected to a simple 35 mm automatic camera (equipped with fixed great angular objective and automatic flash), through an electric wire buried in the ground. The plate is protected by a hermetic plastic bag to avoid damages to the system due to humidity. The circuit inside is opened. When an animal steps on the plate, closes the circuit the camera shoots a photograph. The set device is placed inside a wooden box opened at the front to avoid direct impact by the rain and sun. The camera is set at about 15 cm height (average) above the ground and distanced 2 to 4 meters of the pressure plate. This system is equivalent to the one described by York (1996) and used in Spain by Guzman *et al.* (2002).

▲ **Photograph 3** – Pressure plate camera trap used for lynx detection.

The second photographic system is totally automatic and the photograph shots are activated by differences of intensity of heat. The sensor memorizes the temperature in the detection area and when a heat source makes the temperature vary the camera is activated and a photograph is taken. The sensor is associated to a 35 mm camera with similar characteristics to the one described above. The unit is compact and is protected by a water-resistant container. The sensor was programmed to detect 24 hours/day and once activated a photograph is shot every

20 seconds until the temperature returns to normal. The attractant was distanced 2 to 4 meters from the camera.

2.3.2 Attractants

The attractant scent station consisted in a wooden stake with a piece of cork-tree (*Quercus suber*) bark attached at 40-50 cm above the ground (Guzman *et al.*, 2002). The lure was sprayed on the bark.

The lure used in the scent-stations, associated to the camera-traps, was Iberian lynx urine which, according to Guzmán *et al.* (2002), is considered to be the most effective attractant for the species. A scent lure was preferred instead of food-bait, in order to avoid the deterioration of the station and the attraction of non-target species (which could eat or destroy the bait-station). Another disadvantage of the use of food-bait is the possible contamination of the target species with pathogens due to bait degradation.

Photograph 4 – CamTracker® device used during the current study.▲

The Iberian lynx urine was kindly offered by the Doñana National Park lynx team and was collected from captive specimens held in El Acebuche reproduction centre.

2.3.3 Target areas

The survey areas were selected by combining several factors such as a rabbit density compatible with lynx presence (Palomares, 2001), suitable habitat, proximity to known lynx populations (Cardeña-Andujár and Doñana) and location of potential lynx scats (sent for genetic confirmation) previously collected. By following this protocol the camera-trapping survey area was reduced to Serra da Malcata Natural Reserve (SMNR), Special Protection Area of Moura-Mourão-Barrancos and Vale do Guadiana Natural Park (VGNP).

Each potential study area was surveyed thoroughly with the help of skilled field staff to identify sites that were likely to be visited by lynxes. From among these potential trap-sites, about 50-60 sites, per study area, that showed high suitability for lynx use, were selected for camera trap sampling.

2.3.4 Camera placement

In trapping grid studies, trap spacing is usually established such that all animals moving in the grid interior are exposed to traps (Sanderson, 2002). In this study the cameras were placed in highly suitable areas, that presented the highest probability of being occupied by resident animals.

On the field, cameras were placed at 0.3 to 0.7 km apart (Guzman *et al.*, 2002) on trails and trail intersections and were maintained on the field for at least 28 days, which is the minimum described by Zielinsky *et al.* (1995) for the detection of carnivore species in low densities. A buffer area of half the species home-range (Carbone *et al.* 2001; Karanth & Nichols 2000) was calculated around the camera-traps to represent the total survey area covered by that set of photographic-traps. The effectively sampled area comprises the polygon enclosed by the camera trap locations on the perimeter and a ‘buffer’ area approximately equal in width to half the length of the home range of the animal species being studied (1.5 km) (Ferreras *et al.*, 1997).

2.3.5 Relative abundance of non-target species

Most non-target species cannot be identified as individuals and to estimate their relative abundance we used a relative abundance index (RAI). The number of photos cannot be used to determine population or compare abundance among sites over time. Sunquist & Sunquist (2003) have defined a detection unit of observation, or simply *detection*, as one photograph of a species per camera photo-trap per day (24 hours). If a male and female were detected this would count as two detections. If all photographs are taken of a single species in the course of a day, then this is counted as a single detection.

To compute the RAI for each species, all detections are summed for all camera phototraps over all days, multiplied by 100, and divided by the total number of camera phototrap days. For example, if 12 cameras are run for 180 days, then 12 * 180 = 2160 phototrap days. If 3 male and 4 female lynxes were photographed together each day for 90 days at a site. The lynx RAI would be computed as 7*90*100/(180*12) = 29.17. If a wildcat was photographed 8 times during a single day and twice on separate days, the wildcat's RAI = (1+2)*100/2160 = 0.139.

2.4 Box-trapping

Several methods of trapping have been applied for carnivore species such as padded foot-hold traps and snares and box-traps. Foot-hold traps have been used to capture Iberian lynx by a various number of authors (Castro, 1992; Palomares *et al.,* 2001), however, because of its possibility of inducing serious injuries on the captured species (Phillips, 1996) its use has been forbidden for capturing wild-living animals. Therefore, although box-trapping is not an injury free method it is the safest and commonest method used for capturing these kinds of felids (Palomares *et al.*, 2001; Kitchings & Story, 1984).

The cage-traps used were wire-cages with two guillotine doors activated by a pedal. The dimensions of the traps were approximately 180 × 80 × 80 cm (Photograph 5). A live pigeon was used as bait and was placed at the centre of the cage-trap inside a protection box. Tomahawk® live-traps 1.14 m × 0.38 m × 0.51 m were also used with a similar bait. Traps were disguised with natural vegetation such as *Cistus* spp. and the box floor was covered with dirt and herbaceous species in order to replicate a natural ground cover. Lynx urine was also used as scent lure to increase the capture probability.

As Iberian lynxes tend to use trails to move through their territory, the box-traps were placed in paths and trail intersections.

◄**Photograph 5** – Box-trap used during the trapping campaigns conducted in South-Guadiana.

Traps were visited everyday, at dawn, to avoid the exposure of the captured animals to high temperatures and to prevent an excessive stressful situation that could result in health complications.

Capture techniques were only applied in the South Guadiana valley areas, due to the possibility of capturing dispersing lynxes from the Doñana population.

2.5 Lynx detection probability

In a study of this nature it is crucial for researchers to estimate the detection probability of the target species in the surveyed area. We should take into account the possibility that one or more individuals are present in the surveyed area without being detected, neither by cameras or box-traps. Therefore the “detection probability” should be a reflection of the confidence that we have in the collected data.

During the Iberian lynx census, traps (both cameras and boxes) were placed on the field so that a minimum convex polygon (MCP) was formed by their relative positions. The method was standardized in order that the traps were placed between 300 and 700 meters from each other and the polygon that they formed covered, as much area as possible, of optimal lynx habitat.

As previously described, we determined an external buffer area around the polygons formed by trapping stations. Usually, the buffer width is determined as a function of the species activity or/and its home-range (Karanth & Nichols, 1998). Some authors use this distance as half of the furthest recorded photos of an individual specimen of the target species. Half of the diameter of the species home-range can also be used if the data is adequate (Carbone *et al*, 2002). However, these buffers are usually calculated with the purpose of determining a species density or abundance in the sampled area (Karanth & Nichols, 1998; Carbone *et al*, 2002). This fact means that positive results must be obtained during the survey and that, either we can identify animals individually, or photographic indexes must be directly correlated with the species density. Whatever the case should be, positive records of the target species presence must be collected.

In our study, the main objective is to use the same principle described above to determine the probability of recording the presence of a lynx present in the surveyed area. To attain this objective, random animal movements were simulated, using a similar process to the one described in Carbone *et al* (2002). We try to determine, for each sampled area, the detection probability assuming that one single Iberian lynx was present.

For this methodological approach, we used Iberian lynxes home-ranges sizes and average daily travelled distances from publish data, respecting the Doñana population (Ferreras *et al*, 1997; Palomares *et al*, 2001).

To obtain the total range of home-range areas, we applied the simulation to the smallest home-range described in literature (14.8 km^2)(described by Ferreras *et al*, 1997, for adult resident Iberian lynxes), and the to largest (36.8 km^2) (described by Ferreras *et al*, 1997, for subadult dispersant Iberian lynxes). The same principle was used for the average daily travelled distance. We simulated movements with the smallest and the largest average travelled distance per day described in literature, 6.4 and 8.7 km/day, respectively (Ferreras *et al*, 1997).

Since there was no way to determine the real shape of a possible resident Iberian lynx home-range, we considered it to be a circular area. Although this home-range shape does not represent a real situation, we assumed that no significant differences between values obtained using this method and a real situation should appear.

For this test, we assumed that all trap sets were placed on the field in such a way that the most suitable area for lynx was covered. The correspondence assumed, between trap placement and habitat suitability, allowed us to start every random walk from the centroid of the MCP, which should fall in a supposed core-area of the possible present animal.

We are fully aware that the values of probability obtained lack on statistical consistency, since they were obtained based in highly speculative presupposes, but they serve as reference to the confidence that we may have of this survey results.

All traps placed on the field were geo-referenced and projected in a GIS software (Arcview 3.2 ®). The MCP was drawn for each camera set and the respective centroid was determined. A circular buffer was created to represent a hypothetical Iberian lynx home-range. The following step in our procedure was to simulate several random daily movements within the “virtual home-range” drawn. Seven series of 100 random daily movements were simulated to determine the daily capture probability. For each set of 100 simulations the total number of transects that intercepted a 5 meter buffer around each trap was considered as a detection (we considered every transect that passed closer than 5 meters to a camera resulted in a positive record due to the scent station effect). The total number of contacts was calculated for the area in question and an average of the seven sets was then calculated for attaining a better confidence to the test results. This average represents the daily detection probability of each set of traps.

For each set of 100 simulations we used the following formula:

$$DP = \Sigma\ PC / 100$$

$$ADP = \Sigma\ DP / N$$

$$TDP = ADP \times NAD$$

Where:

PC – Positive records
DP – Daily detection probability
N – Total number of 100 sets of simulations
ADP – Averaged detection probability
TDP – Total detection probability