

1 **A new insight for monitoring ungulates: Density Surface Modelling of roe deer in a**  
2 **Mediterranean habitat**

3 Ana M. Valente<sup>1</sup>, Tiago A. Marques<sup>2,3</sup>, Carlos Fonseca<sup>1</sup>, Rita Tinoco Torres<sup>1</sup>

4  
5 <sup>1</sup>Departamento de Biologia & CESAM, Universidade de Aveiro, Campus de Santiago,  
6 3810-193 Aveiro, Portugal

7 <sup>2</sup>Centre for Research into Ecological and Environmental Modelling, The Observatory,  
8 University of St Andrews, St Andrews KY16 9LZ, Scotland

9 <sup>3</sup>Centro de Estatística e Aplicações da Universidade de Lisboa, Faculdade de Ciências da  
10 Universidade de Lisboa, Bloco C6 - Piso 4, Campo Grande 1749-016 Lisboa, Portugal

11  
12 Corresponding author:

13 Rita T. Torres

14 [rita.torres@ua.pt](mailto:rita.torres@ua.pt)

15 Telephone: +351234247138

16 **Acknowledgments**

17 We are grateful to all the people who provided valuable assistance in the field. Likewise,  
18 several institutions provided invaluable support: Nature and Forestry Conservation Institute  
19 and especially Núcleo Florestal de Bragança. We would like to thank University of Aveiro  
20 (Department of Biology) and FCT/MEC for the financial support to CESAM RU  
21 (UID/AMB/50017) through national funds and, where applicable, co-financed by the  
22 FEDER, within the PT2020 Partnership Agreement. TAM is partially funded by FCT,  
23 Fundação para a Ciência e a Tecnologia, Portugal, through the project  
24 UID/MAT/00006/2013.

25 **Abstract**

26 Ungulates are especially difficult to monitor and population estimates are challenging to  
27 obtain, nevertheless such information is fundamental for effective management. This is  
28 particularly important for expanding species such as roe deer (*Capreolus capreolus*), whose  
29 populations dramatically increased in number and geographic distribution over the last  
30 decades. In an attempt to follow population trends and assess species ecology, important  
31 methodological advances were recently achieved by combining line or point sampling with  
32 Geographic Information Systems (GIS). In this study, we combined density surface  
33 modelling (DSM) with line transect survey to predict roe deer density in northeastern  
34 Portugal. This was based on modelling pellet group counts as a function of environmental  
35 factors while taking into account the probability of detecting pellets and conversion factors  
36 to relate pellet density to animal density. We estimated a global density of 3.01 animals/100  
37 ha (95% CI: 0.37 - 3.51) with a 32.82% CV. Roe deer densities increased with increasing  
38 distance to roads as well as with higher percentage of cover areas and decreased with  
39 increasing distance to human populations. This recently developed spatial method can be  
40 advantageous to predict density over space through the identification of key factors  
41 influencing species abundance. Furthermore, surface maps for subset areas will enable to  
42 visually depict abundance distribution of wild populations. This will enable the assessment  
43 of areas where ungulate impacts should be minimized, allowing an adaptive management  
44 through time.

45

46 **Keywords:** *Capreolus capreolus*, Iberian Peninsula, distance sampling, density surface  
47 models, GAM

## 48 **Introduction**

49 Large herbivores are particularly difficult to monitor (Schroeder et al. 2014) and ecologists  
50 are continuously searching more robust and precise techniques. Successful strategies for the  
51 management of wide-ranging species require reliable information on density and population  
52 trends (Marques et al. 2001). To cope with the dramatic expansion of ungulates in Europe  
53 and North America over the last decades, effective monitoring programs are pivotal (Rooney  
54 2001; Apollonio et al. 2010). Throughout the last years, significant efforts have been made  
55 to improve the methods used for monitoring wild populations (Buckland et al. 2001; Hedley  
56 and Buckland 2004; Thomas et al. 2010). Distance sampling (Buckland et al. 2001) is  
57 recognised as one of the most robust methods for accounting for uncertain detection  
58 (Buckland et al. 2001; Marques et al. 2007) and it has been shown to be a reliable and robust  
59 method to estimate deer abundance (Marques et al. 2001; Acevedo et al. 2008; Valente et al.  
60 2014). Basically, distance sampling methodology relies on the search for animals or animal  
61 signs from lines or points; for each observation the perpendicular distance from the transect  
62 is recorded and a detection function is estimated, enabling abundance and density estimation  
63 of the population of interest by accounting for undetected animals (or animals signs). With  
64 the fast advance of the spatial analysis techniques, the combination of spatial modelling with  
65 Geographic Information Systems (GIS) on population density estimation has been recently  
66 developed. This was firstly reviewed by Buckland et al. (2000), Hedley et al. (2004) and  
67 Hedley and Buckland (2004) who developed methods for improving abundance estimation  
68 of wildlife taking into account the population's spatial distribution. This has allowed to  
69 include heterogeneity in the population spatial distribution while accounting for the  
70 probability of detecting the animal or its signs. An important output of such approach is a  
71 map with the spatial abundance distribution of a population, which is extremely useful to

72 wildlife managers, particularly when communicating results to non-experts stakeholders  
73 (Katsanevakis 2007; Miller et al. 2013a). The recent development of density surface models  
74 (DSM) enabled the identification of meaningful ecological variables that can affect animal  
75 population's densities (Katsanevakis 2007; Miller et al. 2013a). DSMs offer a robust  
76 estimation of abundance (Katsanevakis 2007) and are simple to integrate within the line  
77 transect framework of distance sampling. Furthermore such models are less dependent on a  
78 random survey design or a uniform habitat coverage and allow the estimation of abundance  
79 in sub-areas of interest, through numeric integration under the section of the fitted density  
80 surface (Katsanevakis 2007). This spatial methodology can also improve management plans,  
81 since it makes possible to identify subtle impacts on species, by estimating spatial  
82 redistribution of animals as a result of a particular hazard (Petersen et al. 2011). DSMs are a  
83 model-based approach corrected for uncertain detection via a distance sampling framework  
84 (Hedley and Buckland 2004; Miller et al. 2013a), being typically implemented via  
85 generalized additive models (GAMs) (Hastie and Tibshirani 1990). DSMs have been  
86 successfully implemented in a few species, *e.g.* aquatic molluscs (Katsanevakis 2007),  
87 marine mammals (Henrys 2005; Burt and Paxton 2006), seabirds (Buckland et al. 2012) and  
88 only recently in ungulate species (Schroeder et al. 2014; La Morgia et al. 2015).

89         The European roe deer (*Capreolus capreolus*) is the most abundant and widespread  
90 cervid species in Europe, with an estimated population of 10 million individuals (Apollonio  
91 et al. 2010). In Portugal roe deer occurs at low densities (Valente et al. 2014) particularly  
92 when compared with central and northern Europe (Apollonio et al. 2010). Following the  
93 current European trend, roe deer density is expected to increase considerably in Portugal  
94 (Torres et al. 2015). It is therefore timely to implement management strategies that can  
95 prevent the potential negative impacts deer can have in the ecosystems, such as traffic car

96 collisions, diseases transmission, impacts on commercial forestry and crop production,  
97 conflicts among deer and human populations, amongst others (for a review see Putman et al.  
98 2011).

99         We combined line transect sampling with spatial analysis to predict the abundance  
100 of roe deer in northeastern Portugal. This was achievable taking into account a set of  
101 environmental variables relevant to the ecology of roe deer. The chosen variables were  
102 human disturbance (distance to the nearest road and distance to the nearest human  
103 settlement) which may be considered analogue to predation risk (Hewison et al. 2001; Torres  
104 et al. 2011) and availability of cover areas, which is particularly important since roe deer is  
105 one of the main prey for Iberian wolf (*Canis lupus signatus*). The abundance predictions  
106 were based on the relationship between pellet groups and environmental factors, taking into  
107 account the probability of detecting pellets while also using appropriate factors to convert  
108 pellet groups abundance into deer abundance. This was done through the collection of  
109 distance data regarding pellet groups along line transects covering the whole survey area.  
110 We expect that the use of such an approach will improve the accuracy of density and  
111 abundance estimates when compared with traditional distance sampling, since it models part  
112 of the spatial variability (Hedley et al. 2004).

113         Indirect methods have already been described in the context of deer populations  
114 (Marques et al. 2001; Acevedo et al. 2008; Valente et al. 2014), however they have never  
115 been used in conjugation with DSM. Although this type of approach have the main drawback  
116 of requiring production and decay rates to convert pellets density in animal's density (which  
117 are not typically easy to obtain – for more details see *Discussion* section), they also provide  
118 several advantages since the field work is easy to carry out - it can be performed by park  
119 rangers to ensure a continuity of data - and results are unbiased even in woodland areas -

120 such as our study area, where direct methods are often not feasible or potentially biased  
121 (Marques et al. 2001; Scott et al. 2002; Anderson et al. 2012). DSM can be applied to other  
122 animals for which pellet group count methods are used to estimate their abundance.  
123 Examples include mountain hares (Newey et al. 2003), elephants (Barnes et al. 1995; Olivier  
124 et al. 2009) and a number of other large vertebrates (Hill et al. 1997; Acevedo et al. 2008;  
125 Carvalho et al. 2013). The methodology is equally applicable to surveys of nests or other  
126 signs for which production and decay rates can be estimated, *e.g.* apes are most easily  
127 monitored by surveying their nests (Plumptre 2000).

128 This study aims to (1) use an indirect methodology to model the density surface of  
129 roe deer in northeast Portugal; (2) estimate the density and abundance of this species, (3) to  
130 relate its density to environmental factors and (4) to compare the results of conventional  
131 distance sampling with density surface modelling.

132

## 133 **Methods**

### 134 *Study area*

135 The study was carried out in northeast Portugal (Montesinho Natural Park – MNP – and  
136 *Serra da Nogueira* – SN) (6°30'–7°12'W, 41°43'–41°59'N and 6°50'–6°56'W, 41°38'–  
137 41°48'N respectively), part of the European Union's Natura 2000 Network, covering an area  
138 of 63,500 ha (Fig. 1). The terrain consists of rolling hills with elevation ranging from 438 to  
139 1,481m. The climate is mainly Mediterranean. The vegetation is diverse, characterized  
140 mainly by oak (*Quercus pyrenaica*, *Q. rotundifolia*, *Q. suber*), sweet chestnut (*Castanea*  
141 *sativa*) and maritime pine (*Pinus pinaster*). The shrub vegetation is dominated by heather  
142 (*Erica* spp.), gum rockrose (*Cistus ladanifer*) and furze (*Ulex europaeus* and *Ulex minor*).  
143 Other mammals present are the Iberian wolf (*Canis lupus signatus*), red fox (*Vulpes vulpes*),

144 wild cat (*Felis silvestris*), wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*), among  
145 others. The study area is crossed by some rivers and includes small villages with a low  
146 human presence (9.5 people per km<sup>2</sup>).

147

#### 148 *Line transects and field work*

149 The survey area was divided in 3 geographic strata: *Serra de Montesinho* (SM: 24,400 ha),  
150 *Lombada National Hunting Area* (LNHA: 20,800 ha) (both inside MNP) and *Serra da*  
151 *Nogueira* (SN: 18,300 ha) (Fig. 1). This was done to improve the precision of the final  
152 density estimate, taking into account a previous study (Valente et al. 2014), which includes  
153 a smaller sample of the same study area (without spatial modelling). This was also done for  
154 management purposes, since a large variation is expected in densities across strata. However,  
155 a common detection function was built pooling the data across the three regions. Transect  
156 location and orientation was randomly chosen, ensuring that they were representative of all  
157 habitat types in the study area. In total, 65 different transects were considered: 22 transects  
158 in SM, 16 in SN and 27 in LNHA. Each transect was 1,000m long: to maximize spatial  
159 coverage and to mitigate sampling dependence, sampling plots consisted of 4 100m on effort  
160 segments, each separated by 200m off effort segments, resulting in a total of 400m on-effort  
161 *per* transect. Later the transects were used to model the detection function and the segments  
162 to perform the density surface modelling. Given practical and logistic constraints precluding  
163 surveying the entire survey area in a single year, field work was conducted in 2012 and 2013  
164 (2012: January and November; 2013: January, February and October), randomly carried  
165 among the three study areas. For modelling the detection function, distance data was pooled  
166 across years and regions. The transects were conducted on foot. A handheld Global  
167 Positioning System (GPS) unit and a compass were used to follow a straight line. A rope

168 was used to facilitate the progress in a straight line, ensuring the scanning of 1 meter from  
169 each side of the line, and guaranteeing accurate distance measurements. The choice of 1  
170 meter width (on each side of the rope) transects was based on Marques et al. (2001), where  
171 the use of long (>50 meters) and narrow transects was suggested to ease the search for pellets  
172 groups in low deer density areas, as is the case for our study area (Valente et al. 2014). The  
173 perpendicular distance from the centre of the group to the transect line was recorded for each  
174 pellet group detected. Additionally, three observation level covariates thought to influence  
175 detectability of pellets (Marques et al. 2007) were recorded: i) the size of the pellet group  
176 (medium, 10 - 40 pellets vs. large, > 40 pellets); ii) dispersion of the group (aggregated vs.  
177 scattered); and iii) type of habitat around the pellet group (open vs. closed). To minimize  
178 bias we considered only pellet groups with ten or more individual pellets (produced at the  
179 same defecation event, identified for similar size, shape, texture and colour). This practice  
180 reduces the risk of counting one spread pellet group as two (Marques et al. 2001).

181

182 *A two-stage approach:*

183 *Modelling the detection function*

184 Distance sampling allows uncertain detection of animals/objects (Buckland et al. 2001;  
185 2004). A detection function,  $g(x)$ , is used to model the decrease in detectability with  
186 increasing distance, from the observer (Buckland et al. 2001; Miller et al. 2013a). The  
187 detection function represents the probability of detecting an object given it is at distance  $x$   
188 from the transect line. The probability of detection for the covered area is then given by:

189

$$P = \int_0^w g(x)\pi(x)dx$$

190



191 where  $w$  is a truncation distance and  $\pi(x)$  represents the distribution of available distances,  
192 assumed to be uniform by design. Formally, this corresponds to the expected value of the  
193 detection function with respect to the available distances. In the first stage we used the  
194 *Distance* package (Miller 2014) in R (R Development Core Team 2013) to estimate roe deer  
195 density and abundance. The global density ( $D$ ) estimate is obtained as a weighted average of  
196 stratum specific estimates, with stratum's areas as weights, *i.e.*

$$197 \quad \hat{D} = \frac{\sum_{i=1}^3 \hat{D}_i A_i}{\sum_{i=1}^3 A_i}$$

198 Three key functions were tested: uniform, half-normal and hazard-rate with the three  
199 adjustment terms available (cosine, simple polynomial and hermite polynomial). The unit  
200 considered for analysis was 400m. The effect of observation level covariates in pellet group  
201 detectability was assessed through Multiple Covariate Distance Sampling (MCDS) analysis  
202 (Marques et al. 2007). Detection function choice was based on the Akaike information  
203 criterion (AIC, Akaike 1974), aided by visual inspection of the histogram of distance data  
204 and goodness-of-fit tests (Burnham et al. 2004). Distance data were right-truncated to  
205 remove 5% of the perpendicular distances as recommended by Marques et al. (2001),  
206 resulting in a maximum width of 95 cm of effective prospection. Density surface modelling  
207 results are based on the most parsimonious detection function obtained in this first stage.

208

#### 209 *Density surface modelling (DSM)*

210 The second stage was also performed in R (R Development Core Team 2013) using the  
211 package *dsm* (Miller et al. 2013b). Modelling of density was implemented at the 100m  
212 segment level, totalling 260 segments. Four segment level spatial covariates were collected  
213 through ArcMAP (version 10.1) and used to model the density surface of roe deer in our  
214 study area: i) geographic coordinates (latitude and longitude); human disturbance variables

215 ii) distance to the nearest road – dist\_road – and iii) distance to the nearest human settlement  
 216 – dist\_hum, and iv) percentage of cover areas (ca\_perc: coniferous and deciduous forests).  
 217 Geographic coordinates and human disturbance variables were collected in the center of the  
 218 100m segments. The percentage of cover areas was extracted in a 1.26 km radius around the  
 219 center of each segment. This represents a home range scale calculated based on home range  
 220 values for Portugal (Carvalho et al. 2008). We used GIS to build the buffers from the center  
 221 of the 100m segments. Land cover information was obtained through CORINE Land Cover  
 222 2006 (CLC2006).

223 The count method of Hedley and Buckland (2004) was applied, using the number of  
 224 pellet groups in each segment as the response variable in the density surface model,  
 225 according to:

$$226 \quad E(n_j) = \hat{p}_j A_j \exp \left[ \beta_0 + \sum_k f_k(z_{jk}) \right] \text{ (Miller et al. 2013a),}$$

227 where  $z_{jk}$  is the value of covariate  $k$  in segment  $j$ ,  $f_k$  represents the smooth function of the  
 228 spatial covariate  $k$  and  $\beta_0$  is an intercept term.  $A_j$  is the segment area and  $\hat{p}_j$  the detection  
 229 probability (if this parameter is constant throughout the segments it will simply be replaced  
 230 by  $\hat{p}$ ). The number of pellets (response variable) for each segment was related to the  
 231 predictor variables through Generalized Additive Models (GAMs) (Hastie and Tibshirani  
 232 1990): a quasipoisson distribution and a logarithmic link function were used. The optimum  
 233 degree of smoothing was defined through Generalized Cross Validation (GCV) score. By  
 234 default *dsm* package applies a factor  $\gamma = 1.4$  to model the effective degree of freedom in the  
 235 GCV score to avoid overfitting (Miller et al. 2013b). The choice of the density surface model  
 236 among the set of candidates was based on the lowest GCV value (Wood 2006), while

237 accounting for the deviance explained by each model as well as the *p-value* of each spatial  
238 variable.

239

#### 240 *Abundance estimation*

241 A prediction grid with 635 square cells of 100ha each was built in ArcMAP (version 10.1).

242 The abundance of roe deer in the study area was estimated as the sum of the estimated

243 abundance in each one of the grid cells,  $E[\hat{n}_r]$ ,  $\hat{N} = \sum_r E[\hat{n}_r]$ , relying on the spatial model

244 chosen for inference. Based on the predictions inferred by the density surface model, and

245 taking into account the value of each variable in each grid cell, an abundance map for the

246 survey area was drawn in R (R Development Core Team 2013). To estimate the abundance

247 two conversion factors were used: i) the decay rate (*i.e.* number of days a pellet group takes

248 to decompose – a pellet group was only considered to have less than six individual pellets),

249 estimated by Torres et al. (2013) for our study area and species of interest ( $176 \pm 31$  days),

250 and ii) the production rate (*i.e.* the number of pellet groups produced by an individual *per*

251 day), calculated in the UK, which was considered to be 20 pellet groups *per* day (Mitchell

252 et al. 1985). These values were embedded in the model through the use of an offset, to

253 convert pellet groups density to animal density, accounting for the variance of the former via

254 a bootstrap procedure and ignoring the non-available variance for the latter (see discussion),

255 allowing a straightforward interpretation of the results. Variance for the abundance estimates

256 of DSM analysis was obtained through the variance propagation method described by

257 Williams et al. (2011). This approach enables a prompt variance estimate for both the global

258 and sub-areas density estimates.

259

260 **Results**

261 *The first stage: Modelling the detection function*

262 Over the 26,000m on effort (SM – 8,800m; LNHA – 10,800m; SN – 6,400m) a total of 365  
263 pellet groups were recorded. The detection function that better fitted the distance data among  
264 the set of candidates was the uniform key function with one cosine adjustment term (Fig. 2).  
265 As expected, the probability of detecting pellet groups decreased with increasing distance  
266 from the line, presenting however a broad shoulder (see discussion) with a surprisingly large  
267 number of observations very close to the transect line (Fig. 2). The three detection functions  
268 that included observation level covariates in the analysis had less support from the data, thus  
269 were discarded for the subsequent analysis (with the three covariates tested – habitat, size  
270 and shape with  $\Delta AIC$  of 2.86, 2.65 and 2.81 respectively). The probability of detection for  
271 the chosen detection function was  $\hat{p} = 0.623 \pm 0.026$  SE.

272

273 *The second stage: Density surface modelling*

274 From all the candidate density surface models, two were selected based on their GCV  
275 score (dsm 1 and dsm 3) (Table 1). The implementation of two DSM's was deemed  
276 necessary to fully exploit the data: a DSM for the analysis of environmental data (DSM  
277 without geographical variables – dsm 1 - with dist\_hum, dist\_road and ca\_perc spatial  
278 covariates), and a DSM that enables a more robust estimate of abundance through the  
279 inclusion of geographical data (DSM with geographical variables – dsm 3 - with dist\_hum,  
280 ca\_perc, latitude and longitude spatial covariates). This division was merely practical, to  
281 ensure the identification of potential impacts of environmental variables, that could be  
282 hidden by the geographical data (taking into account the increase in explained deviance when  
283 these variables were included). Fig. 3 shows the smoothed spatial covariates used in the

284 model without geographical variables, being *dist\_hum* the most important variable in the  
285 analysis as revealed by p-values (Table 1).

286

### 287 *Abundance estimation and uncertainty analysis*

288 The conventional design based distance sampling density estimate was 3.53 animals *per* 100  
289 ha (95% IC: 2.07 – 4.79), with  $\hat{N} = 2,233$  animals, and a CV of 24.30% (Table 2).

290 According to the best density surface model (DSM with geographical variables) the  
291 abundance of roe deer in our study area was estimated to be  $\hat{N} = 1,909$  animals with a density  
292 of 3.01 animals *per* 100 ha (95% IC: 0.37 – 3.51) and a CV of 32.82%. In accordance with  
293 the DSM with geographical variables chosen for inference the distribution map of roe deer  
294 throughout the study area is shown in Fig. 4.

295 The values of abundance, density, 95% confidence intervals and coefficient of  
296 variation (%) of traditional distance sampling and density surface models are shown in Table  
297 2.

298

## 299 **Discussion**

300 Wildlife managers and ecologists are continuously searching for accurate and unbiased  
301 methods to estimate species abundance, density and distribution. Such demand is particularly  
302 difficult for large herbivores (Schroeder et al. 2014) dwelling forested habitats (La Morgia  
303 et al. 2015). Density surface models, by combining animal density spatial variation with  
304 traditional line transect surveys open new possibilities for this (Schroeder et al. 2014).  
305 Estimating densities and relating them to meaningful ecological variables represents a step  
306 further on wildlife management. DSM allowed us to assess population ecological

307 requirements through the predicted species distribution. Our results show that roe deer have  
308 higher densities in areas further away from roads. Previous authors have described a similar  
309 pattern for this species (Hewison et al. 2001; Torres et al. 2012a). Roads are known sources  
310 of disturbance and ultimately can lead to direct mortality events. Roe deer tendency to avoid  
311 roads may be related to the risk of collision, which can jeopardize individual's survival, as  
312 evidenced in red deer (*Cervus elaphus*) (Rowland et al. 2000). Our results evidenced that  
313 roe deer densities increase in areas near human settlements. This is contrary to previous  
314 studies elsewhere (Hewison et al. 2001; Coulon et al. 2008), but also for our study area  
315 (Torres et al. 2012b). Nevertheless, methodological differences might explain these on first  
316 sight puzzling differences. Torres et al. (2012b) used presence/absence of roe deer pellet  
317 groups as an index of habitat use while we estimate actual density for each grid cell, using  
318 additional information and hence potentially more accurate. The increasing density towards  
319 human settlements can be explained by rural depopulation in MNP throughout the last years  
320 (Afonso 2012), resulting in small villages with very low human density. Furthermore the  
321 rural depopulation experienced in MNP leads to land abandonment with consequent plant  
322 regeneration that represent new food resources to deer (Vingada et al. 2010). In our study  
323 area, higher roe deer densities correspond to areas with higher percentage cover. This hints  
324 towards the importance of these areas, particularly for a prey with a hiding strategy. Some  
325 studies (Mysterud and Østbye 1999) suggest that canopy cover functions as part of an anti-  
326 predator strategy, providing hiding places and reduced scent spreading, hence reducing  
327 detection by Iberian wolf.

328 Effectively, as noticed by Katsanevakis (2007) (with *Pinna nobillis*) density surface  
329 modelling - contrarily to the non-spatial conventional distance sampling - provided insights  
330 into ecological patterns that may be the first step to further studies regarding the studied

331 species. In general, the underlying ecological assumptions of the density surface models, as  
332 well as the surface map predicted, fits the data observed during the field survey and previous  
333 studies (Torres et al. 2011; Valente et al. 2014). The survey was conducted over a two year  
334 period. Therefore, the estimated density, represents the average density over the  
335 corresponding time period. The detection function presented a broad shoulder and the  
336 expected decline with distance. With objects of interest like pellets, the main distance  
337 sampling assumptions naturally hold. Our only concern related to the surprisingly large  
338 number of very small distances, which could be due to some specific form of measurement  
339 error. Reassuringly, the estimated detection function appears to be fairly insensitive to these  
340 detections, largely due to the otherwise broad shoulder present. Regarding the CV of the  
341 chosen DSM, it showed an acceptable value, ensuring the predictive power of the survey  
342 method. The predictive power was boosted through the addition of geographical coordinates,  
343 which increased considerably the deviance explained by the spatial variables. The increased  
344 predictive power of the models allows the detections of trends in wild populations with less  
345 field data, which contributes to the feasibility of the methodology (La Morgia et al. 2015).  
346 Contrarily to what was a priori expected, due to accounting for part of the spatial variability,  
347 as suggested by Katsanevakis (2007), the inclusion of the spatial variables in the DSM did  
348 not decrease the variance of the estimate. Effectively, this has occurred in several studies  
349 considering DSMs (Cañadas and Hammond 2006; Katsanevakis 2007; Schroeder et al.  
350 2014), suggesting that other spatial variables might have been helpful to explain spatial  
351 variation in our study area. This deserves further consideration in future studies, since it  
352 could potentially lead to more precise estimates. We should note that bias in density  
353 estimates will arise if the conversion factors considered (decay rate and production rate) are  
354 not valid for our survey. It is expected minimal bias from the decay rate since it was available

355 from our survey region and species (Torres et al. 2013). Since decay can vary across habitats,  
356 the use of a site-specific value for each dominant habitat instead of a mean value could be  
357 assessed in future work. In fact, due to logistical constraints it was not possible to use the  
358 specific value in this work. Nevertheless, we do not believe that was a major limitation in  
359 our study. The key problem with our estimate is the use of a production rate obtained in the  
360 UK over 30 years ago (Mitchell et al. 1985). Furthermore, the value used does not have  
361 corresponding precision measures, which means that the reported density estimate variance  
362 ignores a potential source of variation. However, a clear advantage of the modular form of  
363 the estimator used is that, as soon as a production rate and corresponding standard error are  
364 obtained for our region, the density estimates could be easily updated. Obtaining such  
365 production rate should be a major goal for the effective management of these populations  
366 (Valente et al. 2014).

367         Moreover DSM results need to be carefully interpreted since GAMs model selection  
368 is still a research area under development (Williams et al. 2011; Miller 2014). Effectively,  
369 other indicators should be investigated during distance data spatial modelling (*e.g.* p-values  
370 associated with covariate coefficients). In our analysis, the p-value of the variables revealed  
371 the inexistence of a significant ecological variable ( $p \leq 0.05$ ) for DSM's with geographical  
372 variables. Furthermore, the deviance explained in both models (dsm 1 with 7.17% and dsm  
373 3 with 17.3%) was not satisfactory. These values lie far beneath other studies applying DSM  
374 (Cañadas and Hammond 2006; Katsanevakis 2007; Schroeder et al. 2014 with 48.7, 33.5  
375 and 50.4 % respectively). This suggests future investigation of additional factors potentially  
376 influencing roe deer densities in our study area. Although slope is not heavily pronounced  
377 on our study area the influence of altitude/elevation on abundance distribution must be  
378 assessed in future works. Furthermore, as mentioned earlier, the interaction with the



379 sympatric red deer (Torres et al. 2014) or with its main predator, the Iberian wolf would  
380 grant these species density to be a suitable predictor variable for roe deer. Additionally, an  
381 analysis incorporating sex and season should be assessed in the future, since differences in  
382 male and female roe deer ecological requirements, and differences in resource availability  
383 throughout the year as shown for other deer species (Thirgood 1995) and as seen by  
384 Schroeder et al. (2014) with *Lama guanicoe*, whose abundance showed a peak in summer,  
385 might be expected. These goals must be achieved with direct methodologies, which should  
386 be linked to DSM in a near future for ungulate populations in Iberian Peninsula.

387         We believe that the approach presented here could be easily applied in other studies,  
388 namely assessing interspecific sympatric relations using one species density as a spatial  
389 variable for the other. This paper presents a major advance due to the use of a promising  
390 methodology applied to an indirect approach widely used for ungulate populations. The use  
391 of these indirect methodologies enable the survey of large forested areas, enabling as well  
392 predictions for adjacent areas where there are no relevant differences. Actually, due to its  
393 simplicity, the field work can be carried out by park rangers ensuring the continuity of data  
394 collection. Furthermore, for an elusive species as roe deer, indirect methodologies  
395 potentially present more reliable results, since it is easier to fulfil all distance sampling  
396 assumptions. Data analysis is rather more complex, with results that however outweigh this  
397 drawback. Furthermore the graphic output of this methodology enables the non-experts to  
398 easily interpret the results through the abundance distribution maps. This will ease  
399 considerably the access to scientific information essential to management plans particularly  
400 useful for expanding species. This work is part of a continued long-term monitoring program  
401 and represents a step further in methodological optimization of recently developed distance

402 sampling techniques, which aims to become the future in population size estimation and  
403 ecological assessment.

404

#### 405 **Funding**

406 This study was co-supported by University of Aveiro (Department of Biology) and  
407 FCT/MEC financially supporting CESAM RU (UID/AMB/50017) through national funds  
408 and, where applicable, co-financed by the FEDER, within the PT2020 Partnership  
409 Agreement. TAM is partially funded by FCT, Fundação para a Ciência e a Tecnologia,  
410 Portugal, through the project UID/MAT/00006/2013.

411

#### 412 **Conflict of interest**

413 The authors declare that they have no conflict of interest.

#### 414 **Compliance with Ethical Standards**

415 This article does not contain any studies with human participants or animals performed by  
416 any of the authors.

#### 417 **References**

418 Acevedo P, Ruiz-Fons F, Vicente J, Reyes-García AR, Alzaga V, Gortázar C (2008)  
419 Estimating red deer abundance in a wide range of management situations in  
420 Mediterranean habitats. *J Zool* 276(1):37–47

421 Afonso R (2012) O Parque Natural de Montesinho e a promoção do desenvolvimento local.  
422 MsC dissertation in Urban and Regional Planning, Aveiro University, Aveiro, Portugal

423 Akaike H (1974) A new look at the statistical model identification. *IEEE Trans Automatic*  
424 *Control* 19:716–723

- 425 Anderson CK, Nielsen CK, Hester CM, Hubbard RD, Stroud JK, Schaubert EM (2012)  
426 Comparison of indirect and direct methods of distance sampling for estimating density  
427 of white-tailed deer. *Wildlife Soc B* 37(1):146-154
- 428 Apollonio M, Andersen R, Putman R (2010) *European ungulates and their management in*  
429 *the 21st century*. Cambridge University Press, Cambridge
- 430 Barnes RFW, Blom A, Alers MPT, Barnes KL (1995) An estimate of the numbers of forest  
431 elephants in Gabon. *J Trop Ecol* 11(1):27-37
- 432 Borkowski J, Ukalska J (2008) Winter habitat use by red and roe deer in pine-dominated  
433 forest. *For Ecol Manage* 255:468-475
- 434 Buckland ST, Goudie IBJ, Borchers DL (2000) Wildlife population assessment: past  
435 developments and future directions. *Biometrics* 56(1):1-12
- 436 Buckland ST, Anderson DR, Burnham KP, Laake JL, Borchers DL, Thomas LT (2001)  
437 *Introduction to Distance Sampling*. Oxford University Press, Oxford
- 438 Buckland ST, Anderson DR, Burnham KP, Laake JL, Borchers DL, Thomas L (2004)  
439 *Advanced Distance Sampling: Estimating Abundance of Wildlife Populations*.  
440 Chapman & Hall, London
- 441 Buckland ST, Burt ML, Rexstad EA, Mellor M, Williams AE, Woodward R (2012) Aerial  
442 surveys of seabirds: the advent of digital methods. *J Appl Ecol* 49:960–967
- 443 Burnham KP, Buckland ST, Laake JL, Borchers DL, Marques TA, Bishop JRB, Thomas L  
444 (2004) Further topics in distance sampling *Advanced Distance Sampling*. University  
445 Press, Oxford
- 446 Burt ML, Paxton CGM (2006) Review of density surface modelling applied to JARPA  
447 survey data. 1–5
- 448 Cañadas A, Hammond PS (2006) Model-based abundance estimates for bottlenose dolphins  
449 off southern Spain: implications for conservation and management. *J Cetacean Res*  
450 *Manag* 8(1):13
- 451 Carvalho P, Nogueira AJA, Soares AMVM, Fonseca C (2008) Ranging behaviour of  
452 translocated roe deer in a Mediterranean habitat: seasonal and altitudinal influences on  
453 home range size and patterns of range use. *Mammalia* 72:89-94

- 454 Carvalho JS, Marques TA, Vicente L (2013) Population status of *Pan troglodytes* verus in  
455 Lagoas de Cufada Natural Park, Guinea-Bissau. PLoS ONE 8(8):e71527
- 456 Coulon A, Morellet N, Goulard M, Cargnelutti B, Angibault JM, Hewison AM (2008)  
457 Inferring the effects of landscape structure on roe deer (*Capreolus capreolus*)  
458 movements using a step selection function. Landsc Ecol 23(5):603-614
- 459 Hastie TJ, Tibshirani RJ (1990) Generalized Additive Models. Chapman and Hall, London
- 460 Hedley SL, Buckland ST (2004) Spatial models for line transect sampling. J Agric Biol  
461 Environ Stat 9:181–199
- 462 Hedley SL, Buckland ST, Borchers DL (2004) Spatial distance sampling models.  
463 In: Advanced distance sampling: estimating abundance of biological populations.  
464 Oxford University Press, New York, pp 67-89
- 465 Henrys BP (2005) Spatial distance sampling modeling of cetaceans observed from platforms  
466 of opportunity. MSc Dissertation. University of St. Andrews, Scotland
- 467 Hewison AJ, Vincent JP, Joachim J, Angibault JM, Cargnelutti B, Cibien C (2001) The  
468 effects of woodland fragmentation and human activity on roe deer distribution in  
469 agricultural landscapes. Can J Zool 79(4):679–689
- 470 Hill K, Padwe J, Bejyvagi C, Bepurangi A, Jakugi F, Tykuarangi R, Tykuarangi T (1997)  
471 Impact of hunting on large vertebrates in the Mbaracayu Reserve, Paraguay. Conserv  
472 Biol 11(6):1339-1353
- 473 Katsanevakis S (2007) Density surface modelling with line transect sampling as a tool for  
474 abundance estimation of marine benthic species: The *Pinna nobilis* example in a marine  
475 lake. Mar Biol 152:77–85
- 476 La Morgia V, Calmanti R, Calabrese A, Focardi S (2015) Cost-effective nocturnal distance  
477 sampling for landscape monitoring of ungulate populations. Eur J Wildl Res 61(2):285-  
478 298
- 479 Marques FFC, Buckland ST, Goffin D, Dixon CE, Borchers DL, Mayle BA, Peace AJ (2001)  
480 Estimating deer abundance from line transect surveys of dung: sika deer in southern  
481 Scotland. J Appl Ecol 38:349–363
- 482 Marques TA, Thomas L, Fancy SG, Buckland ST (2007) Improving estimates of bird density  
483 using multiple-covariate distance sampling. Auk 124:1229–1243

484 Mayle BA, Peace AJ, Gill RM (1999) How many deer? A field guide to estimating deer  
485 population size. Forestry Commission Publications, Edinburgh

486 Miller DL, Burt ML, Rexstad EA, Thomas L (2013a) Spatial models for distance sampling  
487 data: recent developments and future directions. *Methods Ecol Evol* 4(11):1001–1010

488 Miller DL, Rexstad EA, Burt ML, Bravington MV, Hedley S (2013b) dsm: Density surface  
489 modelling of distance sampling data. R package version 2.1.3. URL: [http://CRAN.R-](http://CRAN.R-project.org/package=dsm)  
490 [project.org/package=dsm](http://CRAN.R-project.org/package=dsm). Accessed 25 November 2015

491 Miller DL (2014) Distance: A simple way to fit detection functions to distance sampling data  
492 and calculate abundance/density for biological populations. R package version 0.8.0.  
493 <http://CRAN.R-project.org/package=Distance>. Accessed 25 November 2015

494 Mitchell B, Rowe JJ, Ratcliffe P, Hinge M (1985) Defecation frequency in roe deer  
495 (*Capreolus capreolus*) in relation to the accumulation rates of faecal deposits. *J Zool*  
496 207(1):1-7

497 Mysterud A, Østbye E (1999) Cover as a habitat element for temperate ungulates: effects on  
498 habitat selection and demography. *Wildl Soc Bull* 27(2):385-394

499 Newey S, Bell M, Enthoven S, Thirgood S (2003) Can distance sampling and dung plots be  
500 used to assess the density of mountain hares *Lepus timidus*? *Wildlife Biol* 3:185–192

501 Olivier PI, Ferreira SM, Van Aarde RJ (2009) Dung survey bias and elephant population  
502 estimates in southern Mozambique. *Afr J Ecol* 47(2):202-213

503 Plumptre AJ (2000) Monitoring mammal populations with line transect techniques in  
504 African forests. *J Appl Ecol* 37:356–368

505 Petersen IK, Mackenzie M, Rexstad EA, Wisz MS, Fox AD (2011) Comparing pre- and  
506 post-construction distributions of long-tailed ducks *Clangula hyemalis* in and around  
507 the Nysted offshore wind farm, Denmark : a quasi-designed experiment accounting for  
508 imperfect detection, local surface features and autocorrelation. Technical Report 2011–  
509 1, CREEM Technical Report, no. 2011-1, University of St Andrews, St Andrews

510 Putman R, Watson P, Langbein J (2011) Assessing deer densities and impacts at the  
511 appropriate level for management: a review of methodologies for use beyond the site  
512 scale. *Mamm Rev* 41(3):197–219

513 R Development Core Team (2013) R: A language and environment for statistical computing.  
514 R Foundation for Statistical Computing. <http://www.R-project.org>. Accessed 25  
515 November 2015

516 Rooney TP (2001) Deer impacts on forest ecosystems: a North American perspective.  
517 *Forestry* 74(3):201–208

518 Rowland MM, Wisdom MJ, Johnson BK, Kie JG (2000) Elk distribution and modeling in  
519 relation to roads. *J Wildl Manage* 64(3):672-684

520 Schroeder NM, Matteucci SD, Moreno PG, Gregorio P, Ovejero R, Taraborelli P,  
521 Carmanchahi PD (2014) Spatial and seasonal dynamic of abundance and distribution  
522 of guanaco and livestock: insights from using density surface and null models. *PloS*  
523 *ONE* 9(1):e85960

524 Scott D, Bacon P, Irvine J (2002) Management of deer in woodlands - Literature reviews of  
525 woodland design, and techniques for assessing populations and damage. Report to the  
526 Deer Commission for Scotland, Centre for Ecology and Hydrology.

527 Thirgood SJ (1995) The effects of sex, season and habitat availability on patterns of habitat  
528 use by fallow deer (*Dama dama*). *J Zool* 235(4):645-659

529 Thomas L, Buckland ST, Rexstad EA, Laake JL, Strindberg S, Hedley SL, Burnham KP  
530 (2010) Distance software: design and analysis of distance sampling surveys for  
531 estimating population size. *J Appl Ecol* 47(1):5-14

532 Torres RT, Santos JP, Linnell JDC, Virgós E, Fonseca C (2011) Factors affecting roe deer  
533 occurrence in a Mediterranean landscape, Northeastern Portugal. *Mamm Biol* 76:491–  
534 497

535 Torres RT, Virgós E, Santos J, Linnell JD, Fonseca C (2012a) Habitat use by sympatric red  
536 and roe deer in a Mediterranean ecosystem. *Anim Biol* 62:351-366

537 Torres RT, Virgós E, Panzacchi M, Linnell JDC, Fonseca C (2012b) Life at the edge: Roe  
538 deer occurrence at the opposite ends of their geographical distribution, Norway and  
539 Portugal. *Mamm Biol* 77:140–146

540 Torres RT, Santos JP, Fonseca C (2013) Persistence of roe (*Capreolus capreolus*) and red  
541 (*Cervus elaphus*) deer pellet-groups in a Mediterranean mosaic landscape. *Mamm Biol*  
542 9:7–18

543 Torres RT, Santos JP, Fonseca C (2014) Factors influencing red deer occurrence at the  
544 southern edge of their range: A Mediterranean ecosystem. *Mamm Biol* 79(1):52-57

545 Torres RT, Miranda J, Carvalho J, Fonseca C (2015) Expansion and current status of roe  
546 deer (*Capreolus capreolus*) at the edge of its distribution. *Ann Zool Fennici* 52:1-14

547 Valente AM, Fonseca C, Marques TA, Santos JP, Rodrigues R, Torres RT (2014) Living on  
548 the edge: roe deer (*Capreolus capreolus*) density in the margins of its geographical  
549 range. *PloS ONE* 9(2):e88459

550 Vingada J, Fonseca C, Cancela J, Ferreira J, Eira C (2010) Ungulates and their management  
551 in Portugal. *European Ungulates and their management in the 21st century*. Cambridge  
552 University Press, Cambridge, pp 392–418

553 Williams ROB, Hedley SL, Branch TA, Bravington MV, Zerbini AN, Findlay KP (2011)  
554 Chilean blue whales as a case study to illustrate methods to estimate abundance and  
555 evaluate conservation status of rare species. *Conserv Biol* 25(3):526-535

556 Wood SN (2006) *Generalized Additive Models: An introduction with R*. Chapman and  
557 Hall/CRC, Florida

558

559

560

561

562

563

564

565

566

567

568

569

570 **Fig. 1** Location of the study area in the Iberian Peninsula with transects location and  
571 prediction grid in the survey area (**SN** – *Serra da Nogueira*; **SM** – *Serra de Montesinho*;  
572 **LNHA** – Lombada National Hunting Area).

573

574 **Fig. 2** Histogram of distance data of uniform detection function with cosine adjustment term.  
575 Observed distances were right-truncated to eliminate the largest 5% of the distances. The  
576 detection function was fitted to continuous data, not binned data, and hence the histogram  
577 bars cannot be interpreted as probabilities.

578

579 **Fig. 3** Shape of the functional forms of smoothed spatial covariates with the DSM without  
580 geographical variables – **(a) dist\_hum** representing the distance to the nearest human  
581 settlement; **(b) dist\_road** representing the distance to the nearest road and **(c) ca\_perc**  
582 representing the percentage of cover areas (coniferous and deciduous forests).

583

584 **Fig. 4** Abundance distribution map of roe deer throughout our study area based on the DSM  
585 with geographical variables chosen for inference (dsm 3).



**Table 1.** Comparison between GCV score, R-square (adjusted), deviance explained, coefficient of variation (CV) and abundance among DSM's with and without geographical variables, with comparison of p-values and estimated degrees of freedom of each variable.

	p-value	Estimated d.f.	GCV	R-square (adjusted)	Deviance explained (%)	CV (%)	Abundance
<b>Without geographical variables</b>							
<b>dsm 1 *</b>			<b>2.694</b>	<b>0.047</b>	<b>7.17</b>	<b>30.45</b>	<b>1878</b>
<b>dist_hum</b>	<b>0.003</b>	1.661					
<b>dist_road</b>	<b>0.019</b>	1.000					
<b>ca_perc</b>	<b>0.022</b>	1.000					
<b>With geographical variables</b>							
<b>dsm 2</b>			2.561	0.106	17.4	36.07	1926
<b>dist_hum</b>	0.096	2.625					
<b>dist_road</b>	0.577	1.000					
<b>ca_perc</b>	0.084	1.000					
<b>geographic</b>	0.030	6.591					
<b>dsm 3 *</b>			<b>2.535</b>	<b>0.108</b>	<b>17.3</b>	<b>32.82</b>	<b>1909</b>
<b>dist_hum</b>	<b>0.105</b>	2.348					
<b>ca_perc</b>	<b>0.067</b>	1.000					
<b>geographic</b>	<b>0.008</b>	6.643					
<b>dsm 4</b>			2.554	0.082	13.9	32.22	1836
<b>ca_perc</b>	0.120	5.904					
<b>geographic</b>	0.003	6.571					
<b>dsm 5</b>			2.552	0.076	13.1	30.30	1846
<b>geographic</b>	0.002	6.190					

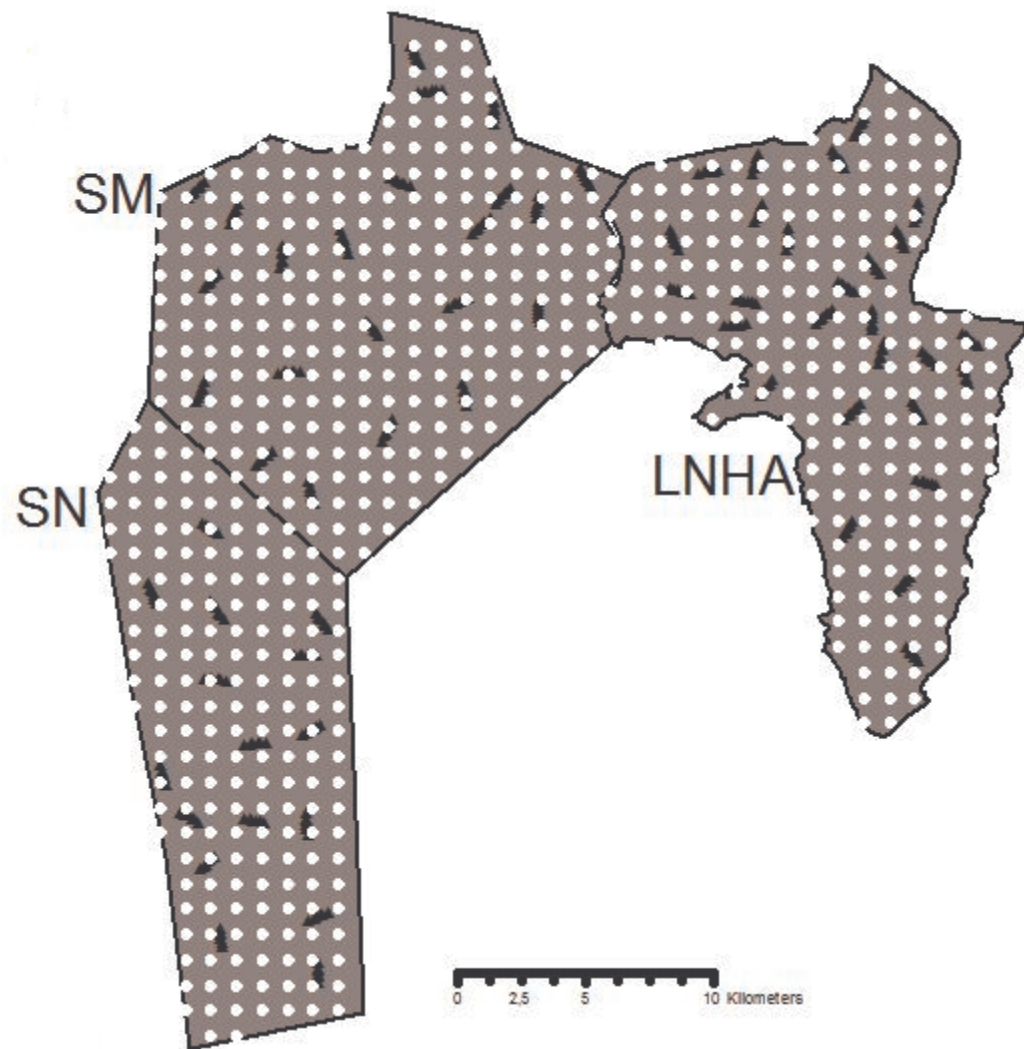
\*dsm chosen for inference.

**Table 2.** Comparison between Density Surface Model and traditional distance sampling through analysis of abundance, density, 95% Confidence Interval and Coefficient of Variation (%) for the total area and for the three sub-areas: SN, SM and LNHA.

Method								
	DSM (with geographical variables)		DS		DSM (with geographical variables)		DS	
	Total area		SN		SM		LNHA	
<b>Abundance</b>	1,909	2,233	662	693	913	1,262	331	278
<b>Density</b>	3.01	3.53	3.62	3.79	3.74	5.17	1.59	1.34
<b>Density - 95% Confidence Interval</b>	0.37 – 3.51	2.07 – 4.79	0.50 – 4.04	2.10 – 6.52	1.67 – 4.40	3.56 – 6.74	0.47 – 3.31	0.82 – 2.51
<b>Coefficient of variation (%)</b>	32.82	24.3	27.90	28.50	27.40	22.54	58.47	32.33



0 45 90 180 Kilometers



0 2.5 5 10 Kilometers

Detection function plot

