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Population density of European wildcats in a pre-alpine area (Northeast Italy) and an assessment of estimate robustness

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Abstract:	Whilst population density is a basic demographic parameter, it is rarely available for the elusive European wildcat, despite its wide distribution. Italy hosts at least five different wildcat populations and little information is available for the wildcats inhabiting the northeast of the Italian peninsula. With the aim to provide the first report on European wildcat population density, we used spatially explicit capture-recapture models applied to camera trapping data in a pre-alpine area in NE Italy. The survey was carried out from May 18th to September 14th, 2015, using 31 camera traps distributed within a 1×1 km grid, placing a single camera per km2. We collected 32 videos of wildcats, corresponding to a total of eleven individuals. Density ± SE estimate was 0.35 ± 0.12 individuals per km2, with the encounter probability (g0) equal to 0.10 ± 0.03, and the spatial scale (σ) equal to 461 ± 62 m, corresponding to a mean home range size of 3.36 km2. In addition, to evaluate our sampling design and the robustness of our estimates we simulated data generation and fitted SECR models under several realistic combinations of number and spacing of detectors, and sampling efforts. Considering the relative standard errors and relative bias our sampling design produced robust estimates, whereas in scenarios with short sampling periods or greater spacing of detectors, the estimates were inadequate. Our study provides previously unavailable data on the biology of the European wildcat from NE Italy and some important considerations concerning sampling design to plan future research.
Response to Reviewers:	Dr. Krzysztof Schmidt comment EDITOR: I have sent your revised paper to one of the reviewers that have previously evaluated your manuscript. Please, see the document uploaded on the Editorial

Manager with all the comments provided by the reviewer. Overall, the reviewer found your paper greatly improved, but still requiring some edits. Please, make sure you follow the comments and suggestions carefully and revise the paper accordingly.
AUTHORS: Thank you for giving us the opportunity to revise the MS again. We accepted all the reviewer suggestions. The paper was also revised by an English native speaker, Crinan Jarrett from the University of Glasgow. Hereafter, you will find a point-by-point response to the major issues, while we simply fixed all the minor issues. We are very sorry for the one-day delay.

Reviewer major comments:
Reviewer #1: I believe that the authors have properly addressed the weak points in the simulation section, but I don't agree with the exclusion of a cat from the population density analysis because its status was not totally clear (i.e. putative hybrid). Overall, I feel that the readability and the English of this revised version are still not satisfactory and that some sentences were not located in the proper sections. I have provided comments for improving these flaws but given that I am not a native English speaker, I would recommend a thorough revision of the manuscript before resubmitting it. I hope the authors can expand their wildcat research in this study area.
Authors: We thank the reviewer for the precious comments and revisions, which helped improve the manuscript. We carried out again the population density analysis and simulations including the cat considered as putative hybrid. The paper was also revised by an English native speaker Crinan Jarrett from the University of Glasgow.

Reviewer minor issues
Authors: We accepted or fixed all the corrections/comments. Please refer to the new MS version for your evaluation.

Population density of European wildcats in a pre-alpine area (Northeast Italy) and an assessment of estimate robustness

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Abstract

Whilst population density is a basic demographic parameter, it is rarely available for the elusive European wildcat, despite its wide distribution. Italy hosts at least five different wildcat populations and little information is available for the wildcats inhabiting the northeast of the Italian peninsula. With the aim to provide the first report on European wildcat population density, we used spatially explicit capture-recapture models applied to camera trapping data in a pre-alpine area in NE Italy. The survey was carried out from May 18th to September 14th, 2015, using 31 camera traps distributed within a 1×1 km grid, placing a single camera per km^2. We collected 32 videos of wildcats, corresponding to a total of eleven individuals. Density ± SE estimate was 0.35 ± 0.12 individuals per km^2, with the encounter probability (g_0) equal to 0.10 ± 0.03, and the spatial scale (σ) equal to 461 ± 62 m, corresponding to a mean home range size of 3.36 km^2. In addition, to evaluate our sampling design and the robustness of our estimates we simulated data generation and fitted SECR models under several realistic combinations of number and spacing of detectors, and sampling efforts. Considering the relative standard errors and relative bias our sampling design produced robust estimates, whereas in scenarios with short sampling periods or greater spacing of detectors, the estimates were inadequate.

Our study provides previously unavailable data on the biology of the European wildcat from NE Italy and some important considerations concerning sampling design to plan future research.

Keywords: *Felis silvestris silvestris*, camera trapping, spatially explicit capture-recapture, sampling design, simulations, secrdesignapp.

Introduction

Once widespread throughout Europe, the European wildcat (*Felis silvestris silvestris* Schreber, 1777) has suffered a severe decline in recent centuries (Nowell and Jackson 1996), which has resulted in its current fragmented distribution (Lozano and Malo 2012). The European wildcat is a protected species included in Annex IV of the European Habitats Directive (92/43/CEE) and in Annex II of the Bern Convention. Currently, the species is classified as being in the "Least concern" category of the IUCN red list of threatened species, with contrasting population trends, i.e., decreasing throughout most of its range (Yamaguchi et al. 2015), but increasing in some European countries (Nussberger et al. 2018). Nevertheless, the species continues to be affected by several threats (Lozano and Malo 2012), in particular habitat fragmentation (Lozano et al. 2007; Klar et al. 2012; Anile et al. 2019; Gil-Sánchez et al. 2020), hybridization with domestic cats (*Felis silvestris catus*; Daniels and Corbett 2003; Pierpaoli et al. 2003; Lecis et al. 2006; Oliveira et al. 2008a, b; Hertwig et al. 2009; Macdonald et al. 2010; Mattucci et al. 2013, 2019; Witzenberger and Hochkirch 2014) and human-induced mortality such as road kill and poaching (Nowell and Jackson 1996; Krone et al. 2008; Devillard et al. 2013; Falsone et al. 2014).

In recent years there has been a considerable increase in the number of studies on the European wildcat, in particular in the field of genetics (Mattucci et al. 2016), morphology (Kitchener et al. 2005; Krüger et al. 2009), trophic ecology (Piñeiro and Barja 2011; Apostolico et al. 2016; Széles et al. 2018) and habitat selection (Monterroso et al. 2009; Lozano 2010; Oliveira et al. 2018; Gil-Sánchez et al. 2020). However, detailed data on European wildcat distribution, reproductive biology, and population dynamics are lacking in most of its range (Lozano and Malo 2012; Lozano et al. 2013). Additionally, some basic demographic parameters may be unknown in a number of areas, such as those that have been colonized recently (Wening et al. 2019). Moreover, sampling wildcats is a challenging task given that the European wildcat is an elusive species (Piñeiro et al. 2012), mainly nocturnal (Daniels et al. 2001; Germain et al. 2008) and is often found at low densities (usually in the range of 0.2-0.3 individuals per km^2; Can et al. 2011; Anile et al. 2014; Gil-Sánchez et al. 2015).

However, by using camera traps researchers can now reliably detect even elusive and rare species (Rowcliffe and Carbone 2008; Rovero et al. 2013), like the European wildcat. In particular, non-invasive capture-mark-recapture (CMR) analysis based on camera traps images of wild cats with natural unique markings can accurately estimate population abundance or density (Karanth 1995; Karanth and Nichols 1998; Anile et al. 2010, 2012a; Can et al. 2011), as well as the recent development of spatial capture-recapture (Kilshaw and Macdonald 2011; Anile et al. 2014; Gil-Sánchez et al. 2015, 2020). Spatially explicit capture-recapture (SECR) models are an extension of CMR models that use the spatial organization of encounters to estimate detection, space use, and density (Efford 2004; Royle et al. 2014). Particularly in studies that assume a closed population (i.e., no birth, death, immigration, or emigration during the sampling period) and that use SECR models, it is essential to obtain sufficient detections in a short time and in a sufficient spatial extent (Ash et al. 2020). For low-density populations or for rare and elusive species this obtainment can be challenging (Karanth and Nichols 1998), so sampling design plays a key role for the reliability of survey results (Royle et al. 2014; Ash et al. 2020; Dupont et al. 2021). Simulations can be used to assess the effectiveness of sampling designs and to avoid unbiased estimates (Obbard et al. 2010; Tobler and Powell 2013; Smith et al. 2020; Ash et al. 2020; Green et al. 2020; Dupont et al. 2021).

The coat colour and marking system of the European wildcat allows individual identification (Ragni and Possenti 1996) and surveys that use camera traps to detect European wildcats have been used in many European countries, e.g., Scotland (Kilshaw and Macdonald 2011; Littlewood et al. 2014; Kilshaw et al. 2015), Spain (Sarmento et al. 2009; Soto and Palomares 2014; Gil-Sánchez et al. 2015), Turkey (Can et al. 2011), the Netherlands (Canters et al. 2005) and Germany (Beutel et al. 2017).

Italy currently hosts two subspecies of wildcat: the north African wildcat *Felis silvestris libyca*, present only on the island of Sardinia, and the European wildcat *Felis silvestris silvestris*. Mattucci et al. (2013) found that the European wildcats in Italy are genetically subdivided into three well-defined clusters and four populations: (a) one in the Friuli Venezia Giulia and Veneto regions (Northeastern Italy), which is connected with the Slovenian and Croatian populations (Lapini 2006), (b) one on the island of Sicily (Pierpaoli et al. 2003); and (c) one in the Central-southern Italian Peninsula (Velli et al. 2015), further split into two subpopulations distributed on the eastern (Apennine Mountains and Hills) and western (Maremma Hills and Lowlands) sides of the Apennine ridge. In addition, the European wildcat population in the north-western Italian peninsula may have been extinct since 1980s (Ragni et al. 2012), despite recent records in Liguria (NW Peninsula) that provide some evidence of possible recolonization in this region (Loy et al. 2019).

Several studies on the European wildcat have been carried out in Sicily (Anile et al. 2009, 2010, 2012b, a, 2014, 2019, 2020) and in the Central-southern Italian Peninsula (Bizzarri et al. 2010; Velli et al. 2015; Veronesi et al. 2016; Anile et al. 2017), but information on the status of the European wildcat in Northeastern Italy is still lacking (Ragni et al. 1989; Lapini 2006; Lapini et al. 2014). The scarce existing literature in this region includes a study by Lapini (2006) describing its distribution, a physiological study by Franchini et al. (2019), and two studies describing its expansion towards the Western Alps (Spada et al. 2014, 2016). There is no reliable or recent information concerning basic demographic parameters such as the population density of the European wildcat for the northeastern Italian Peninsula, or for neighbouring countries Slovenia (Krofel et al. 2021) and Croatia. Hence, our main goal was to assess the population density of the European wildcat in a pre-alpine area located in north-eastern Italy by using camera trapping and spatially explicit capture-recapture models. Moreover, we further evaluated our sampling design and the reliability of our estimates by simulating scenarios in which the number and spatial arrangement of detectors and the length of the survey were modified.

Materials and methods

Study area

The study area extends over 31 km^2 in the Carnic Prealps in the province of Pordenone (centroid coordinates: E 332540, N 5123192 WGS84 UTM zone 33N) (Fig. 1). Elevational range is 425-1148 m a.s.l. and vegetation cover is dominated by forests (66.2% broad-leaved forests and 22.2% mixed forest), with some patches of grassland (5.8%) and shrubland (2.5%) (CORINE Land Cover 2018; European Environment Agency; http://www.sinanet.isprambiente.it). Forested areas are dominated by beech (*Fagus sylvatica*), in association with black hornbeam (*Ostrya carpinifolia*), manna ash (*Fraxinus ornus*), and Norway spruce (*Picea abies*).

Climate is typically continental-temperate (mean yearly temperatures: 11.7 °C) and characterized by abundant precipitation (yearly rainfall 2191 mm). Typically, the ground is covered by snow between December and March, with an average of 36% ± 46 (SD) of the ground covered (period 2000-2020; data obtained from the product "MODIS/Terra Snow Cover Monthly L3 Global" of NASA (Hall and Riggs 2021).

Data collection and wildcat identification

The camera trapping survey was carried out between May 18th and September 14th, 2015, for a total of 120 consecutive days. We used a Tessellation Stratified Sampling design (Morrison et al. 2006; Sutherland 2006) superimposing a 1×1 km grid to our study area. The choice of grid size was based upon previous studies of European wildcat population densities in Italy (Anile et al. 2009, 2010), to ensure captures and recaptures. We placed 31 camera traps (models Scout Guard SG570 and Scout Guard SG550), one camera per site, as close as possible to the cell centres along existing paths (average spacing among traps: 735.74 m; Fig. 1). Each camera was placed on a tree at 40-50 cm above the ground and was checked every two weeks. Cameras were set to an active mode, taking 20-second videos for each movement detected.

Individual identification was conducted using the method proposed by Ragni and Possenti (1996), which has been proven to be highly congruent with genetic studies (Randi et al. 2001; Oliveira et al. 2008b). The coat patterns of the following anatomical regions *gularis*, *occipitalis-cervicalis*, *scapularis*, *dorsalis*, *lateralis*, and *caudalis* (Fig. 2; Ragni and Possenti 1996) were compared. Comparisons were mainly based on (i) the number, shape, dimension, and position of stripes on the trunk and limbs and on (ii) the number and shape of the tail rings. All videos were independently inspected by three authors (FF, MP, and SP) and only concordant individual identifications were included in the analyses (Kelly et al. 2008; Alexander and Gese 2018).

Data analysis

Spatially explicit capture-recapture models (SECR)

The package *secr* (Efford 2015) for the statistical environment R v.4.0.1 (R Core Team 2020) was used to fit SECR models using likelihood inference for estimating population density. Following Zimmermann and Foresti (2016), the entire sampling period was divided into sampling occasions, defined as five consecutive trap-nights, resulting in 24 periods in total. We constructed a 'capthist' object, a matrix that holds spatial capture histories, detector locations and functionality, and occasions, following Zimmermann and Foresti's (2016) R script, although dedicated tool already exist (Niedballa et al. 2016). To check the assumption of a closed population, the Otis et al. (1978) test was performed with 'closure.test' command in R. The models were fitted assuming a half-normal detection function, i.e., trap encounter probability is assumed to decrease with increasing distance from the individual's activity centre, and from proximity detectors (Efford 2011; Zimmermann and Foresti 2016). To select the minimum buffer width and create object masks for SECR models, we followed Pesenti and Zimmermann (2013). We created several masks for 12 buffer widths ranging from 250 to 3000 m, with increments of 250 m. SECR densities were then calculated using each mask created with 'null model' formulation, which assumed constant values for animal density D, baseline encounter probability g_0 and spatial scale σ (Zimmermann and Foresti 2016). Preliminary analysis showed that the estimated densities decreased rapidly with increasing buffer width and stabilized when the buffer was ≥ 1 km: thus, we retained 1 km-buffer in subsequent analyses.

We modelled (i) the density component as *D ~ 1*, indicating density as a constant across trapping occasions; (ii) the spatial scale parameter as *σ ~ 1*, indicating *σ* was fixed as a constant across all individuals; and (iii) the encounter probability g_0 as '*1*' indicating a fixed constant baseline encounter rate, '*t*' and '*T*' indicating time factor and time trend, respectively, '*k*' and '*K*' referring only to site response, i.e., site learned response and site transient response and, '*b*', '*B*', '*bk*' and '*Bk*' indicating changes in behavioural response, i.e., learned response, transient response, site-specific learned response and site-specific transient response (Table 1; Efford 2015). Although no bait was used, we tested the hypothesis that European wildcats changed their behaviour due to biological (Nowell and Jackson 1996) and physiological (Piñeiro et al. 2020) aspects, or due to human presence such as the activity required for camera trap deployment and maintenance (Caravaggi et al. 2020). Following Royle et al. (2014) and Zimmermann and Foresti (2016), we used Akaike's Information Criterion (AIC) to compare models and to select the best fitting models. Models with Δ_iAIC≥2 were discarded (Anderson and Burnham 2002). Estimates of home range size were obtained converting the parameter *σ*, under the assumption of circular bivariate normal distribution (i.e., half-normal detection function; Kilshaw et al. 2015).

Sampling design simulations

We simulated data for several realistic scenarios in which the number and spacing of detectors, as well as the sampling effort (i.e., occasions) varied to evaluate the robustness of our estimates and sampling design. We used our mean estimates of *D*, *g0* and *σ* to generate data in the other scenarios. Simulated data included the combination of detectors and occasions used in our study (i.e., 31 detectors, 1 km spacing and 24 occasions), while also varying the distance between camera traps (800, 900, 1000, 1100 and 1200 m) and the number of cameras (50, 38, 31, 27 and 21) (Fig. 3). Furthermore, in order to determine the influence of sampling effort on estimates, simulated data included scenarios with 8, 12, 16, 20 and 24 occasions, corresponding to 40, 60, 80, 100 and 120 days respectively; the maximum number of occasions was 24, as in our sampling, to respect the population closure assumption. All these combinations resulted in a total of 25 unique scenarios and each was replicated 500 times, obtaining 12,500 datasets generated assuming a homogeneous Poisson distribution of the home ranges.

We specified a buffer width of 4x σ, as recommended by Efford (2019a), and we fit SECR models for each dataset using the half-normal detection function. For each scenario we computed the number of individuals, the number of detections, relative standard error (RSE) and relative bias (RB); scenarios with less than 5 spatial recaptures (i.e., detection of unique individuals at multiple locations in space) were classified as a 'pathological' design (Efford and Boulanger 2019; Smith et al. 2020). We assessed the robustness of our results comparing RSE and RB values associated to our sampling design with those associated to other scenarios. Following Ash et al. (2020), we considered our results robust if our RB was lower than 10% (Efford and Fewster 2013) or not significantly different from the RBs estimated in the other scenarios. Simulations were run in the web-based application 'secrdesignapp' (https://www.stats.otago.ac.nz/secrdesignapp/; Efford 2019b) which uses functions of *secrdesign* R package (Efford 2019a).

Results

European wildcat population density

Camera traps were active for a total of 2990 trap-nights of which 854 (22.2%) were lost due to theft (4 cameras) or due to technical problems such as camera failure or dead batteries. We obtained 32 capture events of European wildcat with a capture rate of 1.07 captures/100 trap-nights. We identified 11 individuals from 15 cameras. Four individuals were detected at two or three cameras (average maximum distance: 1247.25 m) whereas seven individuals were detected at only one camera; only two individuals were detected once, while the remaining nine individuals were detected from one to six times (Fig. 1). Most of the videos were recorded during nocturnal (63%) or diurnal (28%) hours, and few being recorded during crepuscular hours (3% sunrise and 6% sunset).

Ten videos were discarded because the morphological characters were not observable for conducting the individual identification. One individual presented intermediate morphological characteristics between the European wildcat and the domestic cat; we considered it as a putative hybrid, and it was included in the analysis (Fig. 2b).

The result of the Otis et al. (1978) test supported our assumption of a closed population (Z = -0.77, P = 0.22). Model selection indicated that the best fitting model was the constant one ($D\sim 1\ g_0\sim 1\ \sigma\sim 1$), in which the encounter probability was fixed with a constant baseline encounter rate (Table 1). The population density ± SE estimated by the model was 0.35 ± 0.12 individuals per km^2, with parameter g_0 ± SE equal to 0.10 ± 0.03 and parameter σ ± SE equal to 461 ± 62 m, giving a 95% home range radius of 1.03 km and a home range size of 3.36 km^2.

Survey design evaluation

Eleven out of 25 scenarios resulted in a ‘pathological’ design or with very high RSE and RB (ESM Table S1), implying that if these scenarios were used the resulting population density estimate would have been biased. The only non-pathological design with 21 detectors spaced 1200 apart was that with the highest number of occasions. The ‘pathological’ rate of the sampling designs increased in scenarios where the detectors were far apart, and the sampling period was short (ESM Table S1).

In non-pathological designs, the number of individuals (N) and the detections (*ndet*) increased with the number of detectors, number of occasions and detector proximity (Fig. 4a and 4b). The highest N (15) and *ndet* (54.7) were obtained in the scenario with 50 detectors spaced 800 m and for 24 sampling occasions (Fig. 4a and 4b, ESM Table S1). The lowest N (10.9) and *ndet* (20.8) was observed in the scenario with 38 detectors spaced 900 m apart and for 12 sampling occasions (Fig. 4a and 4b, ESM Table S1). The scenario in which we simulated our sampling protocol (i.e., 31 detectors spaced 1000 m apart and 24 occasions) produced 12.9 N and 33.9 *ndet* (Fig. 4a and 4b, ESM Table S1).

The RSE ranged from 27.44% (for the scenario with 50 detectors spaced 800 m apart and 24 occasions) to 39.14% (for the scenario with 38 detectors spaced 900 m apart and 12 occasions) and increased with fewer occasions and when detectors were far apart (Fig. 4c, ESM Table S1). RB ranged from 0.39%, in the scenario with 50 detectors spaced 800 m apart and 16 occasions, to 12.87% for the scenario with 38 detectors spaced 900 m apart and 12 occasions (Fig. 4b). The scenario in which we simulated our sampling design produced a RSE of 31.17% and an RB of 3.66%.

Discussion

In this study we provide the first density estimate of a European wildcat population in Northeastern Italy from a relatively small study area within the Carnic Prealps. This result is important because population density estimates for the European wildcat populations found throughout the biogeographic group of north-eastern Alps and northern Balkan regions (Dinaric Alps; Mattucci et al. 2016) are either scarce, dated (Dimitrijevic 1980), or completely lacking like in Slovenia and Croatia. Population density estimates are crucial parameters for informing the status of a local population, but also as a starting point in the planning of future studies in the Alpine biogeographical region, given the European wildcats expansion towards more western Alpine areas (Spada et al. 2014, 2016).

The density of 0.35 individuals per km^2 that we estimated is in line with previous findings in Italy (0.12-0.46 individuals per km^2; Table 2) and in other countries surrounding the Alpine region (0.10-0.50 ind./km^2; Table 2), however some of these estimates were obtained from study areas with different climate (i.e., Mediterranean vs. Continental; Anile et al. 2012a, 2014) making comparisons difficult.

Our density estimate also falls within the range of values reported in other countries (Table 2). For example, estimates from Kilshaw et al. (2015) are higher (0.68 individuals per km^2), but these include a high percentage of hybrids. Lower values are commonly reported for other areas, which could be due to the large extension of these study areas (studies at a regional scale include large portions of unsuitable habitats; Gil-Sánchez et al. 2020), low food availability (Gil-Sánchez et al. 2015), as well as other environmental factors (Anile and Devillard 2020).

The home range size of the European wildcat presents a wide heterogeneity, with marked variations in particular between sexes (Anile et al. 2017; Maronde et al. 2020). Unfortunately, it was not possible to distinguish sex from camera trap videos, so we calculated the mean home range (3.36 km^2) using the same spatial scale parameter (σ) for all individuals. The same procedure was applied in Kilshaw et al. (2015) and Maronde et al. (2020) obtaining a mean home range of 3.8 and 14.3 km^2 respectively.

Several studies, including ours, have showed that non-invasive camera trapping and SECR analysis are reliable methods for estimating European wildcat population density (e.g., Anile et al. 2014; Maronde et al. 2020; Gil-Sánchez et al. 2020), as well as for other wild cats (Royle et al. 2014; Satter et al. 2019).

Camera trapping capture-recapture studies usually require the combined use of two camera traps per site (Anile and Devillard 2015), but, because of resource limitations, only one camera was deployed per site, a factor which may have affected the accuracy of our estimates (Zimmermann and Foresti 2016). Deploying two camera traps per site to photograph both flanks of an animal facilitates recognition of individuals (Zimmermann and Foresti 2016), but this recognition is still possible using a single camera trap (Anile et al. 2010) and by estimating population density with SECR models (Jędrzejewski et al. 2017). A single camera trap per site may increase the proportion of detections with insufficient quality, hence preventing individual identification, for example, in our study ~25% of the videos containing the species were discarded, which falls in line with the percentage of another study (Pease et al. 2016). Furthermore, based on our experience, recording videos instead of taking images is a more convenient choice as video footage makes it possible to observe animals in movement, showing different diagnostic parts of the body. However, our intention for future studies is to deploy camera traps in pairs at each site to better detect animals.

As described above, collecting data in order to study European wildcats and other elusive species can be challenging (Foster and Harmsen 2012). It is therefore essential to use effective detection methods (i.e., camera trapping) and sampling designs (Sutherland 2006; Royle et al. 2014). In SECR studies it is critical to consider the spatial arrangement and number of detectors because these factors could significantly affect density estimates (Sollmann et al. 2012; Sun et al. 2014). For this reason, in our study we used simulations to evaluate and validate our sampling design and asses if our results were robust. Simulations are a valuable tool for this objective but are currently underutilized because they are laborious and require computer-intensive tasks (Royle et al. 2014; Efford and Boulanger 2019). However, the web-based application ‘secrdesignapp’ (Efford 2019b) is a very intuitive and easy tool and can reduce such difficulties.

Through simulations and comparison against other realistic scenarios, we were able to evaluate the effectiveness of our sampling design and the robustness of our results. Specifically, we investigated how RSE and RB varied according to sampling design, as done in other studies (Kristensen and Kovach 2018; Smith et al. 2020; Ash et al. 2020; Green et al. 2020; Dupont et al. 2021). When considering RSE estimates, we did not observe a high variation among scenarios, even if in the majority the 95% confidence intervals (CIs) did not overlap (Fig. 4c); while the RB was comparable across scenarios and for a major part of these the 95% CIs overlapped (Fig. 4d). The relatively high RSE of our sampling design (ca. 31) was not very different from those produced by other scenarios (min 27.44 - max 39.14) and similar to that estimated by Ash et al. (2020) for a tiger *Panthera tigris* study in Thailand (ca. 30), and may be due to the low population density of the European wildcat. The RB was reasonably low (<10%; Efford and Fewster 2013), indicating that our results are robust and reliable.

Furthermore, by means of simulations we found that the sampling would have been inadequate in many scenarios (i.e., ‘pathological’ designs; 44% of scenarios). Indeed, in cases where a short sampling period or greater spacing of detectors were simulated, either the pathological rate increased or the RSE and RB were higher. Hence, in our case, reducing the sampling effort and increasing the camera spacing to more than 1100 m produced an excessive loss of information, resulting in too few captures and recaptures and biased estimates. This agrees with previous studies (Sollmann et al. 2012; Sun et al. 2014) and suggests that detector spacing is ideally around or within 2x sigma to ensure recaptures, which in our case corresponded to 938 m.

In conclusion, our study could represent a valid contribution to the knowledge base of the ecology of the European wildcat in pre-alpine areas. Future studies should however be conducted at a broader geographic scale and for longer periods to assess population trends and conservation status at the regional level. They should also include some genetic characterization; the detection of a putative hybrid suggested that this population may be affected by hybridization as observed in all Italian wildcat populations (Mattucci et al.

2013). Genetic studies are needed to quantify this risk and, if necessary, to plan conservation actions for the protection of European wildcats.

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Table 1 - The SECR models used to estimate European wildcat density ranked by Akaike's Information Criterion (AIC); In **bold**, the best fitting model.

Models	**Number of parameters**	**Log-likelihood**	**AIC**	**Δ_iAIC**
$D\sim1\ g_0\sim1\ \sigma\sim1$	**3**	**-62.459**	**130.918**	**0.000**
$D\sim1\ g_0\sim K\ \sigma\sim1$	4	-62.459	132.918	2.000
$D\sim1\ g_0\sim bk\ \sigma\sim1$	4	-140.632	289.264	158.346
$D\sim1\ g_0\sim k\ \sigma\sim1$	4	-141.785	291.570	160.652
$D\sim1\ g_0\sim b\ \sigma\sim1$	4	-142.787	293.573	162.655
$D\sim1\ g_0\sim Bk\ \sigma\sim1$	4	-143.473	294.946	164.028
$D\sim1\ g_0\sim T\ \sigma\sim1$	4	-143.619	295.237	164.319
$D\sim1\ g_0\sim B\ \sigma\sim1$	4	-144.033	296.067	165.149
$D\sim1\ g_0\sim t\ \sigma\sim1$	26	-132.332	316.665	185.747

Table 2 - List of studies concerning density estimation on the European wildcat. Densities are shown as average ± standard error or minimum - maximum.

References	**Study area**	**Density (ind./km^2)**	**Methods**
Italy			
Present study	North-eastern Italy (Carnic Pre-Alps)	0.35 ± 0.12	Camera trapping - SECR models
Anile et al. 2012a	Southern Italy (Mt Etna, Sicily)	0.46 ± 0.13 0.28 ± 0.10	Camera trapping - software CAPTURE
Anile et al. 2014	Southern Italy (Mt Etna, Sicily)	0.32 ± 0.10	Camera trapping - SECR models
Velli et al. 2015	Central Italy (Foreste Casentinesi National Park)	0.14 - 0.29	Non-invasive genetic and camera trapping
Bizzarri et al. 2010	Central Italy (Apennines)	0.12	Radio-tracking
Ragni 2006	Central Italy (Central Apennines - Umbria)	0.20 - 0.30	Snow-tracking and radio-tracking
From countries surrounding the Alpine region			
Balzer et al. 2018	Germany	0.10 - 0.50	SECR models
Maronde et al. 2020	Switzerland	0.26	Camera trapping - SECR models
Kery et al. 2011	Switzerland	0.29 ± 0.06	DNA - Bayesian spatial capture-recapture models
Camera trapping studies from other countries			
Gil-Sánchez et al. 2015	Southern Spain (Sierra Nevada)	0.093 ± 0.019	Camera trapping - SCR models
Gil-Sánchez et al. 2020	Southern Spain (Andalucía)	0.069 ± 0.019	Camera trapping - Bayesian SECR models
Matias et al. 2021	Portugal	0.032 ± 0.012	Camera trapping - SCR models
(Dimitrijevic 1980)	Serbia	0.164–0.449	
Can et al. 2011	Western Turkey	0.22 ± 0.06	Camera trapping - software MARK
Kilshaw and Macdonald 2011	Scotland	0.29 ± 0.13	Camera trapping - software SPACECAP
Kilshaw et al. 2015	Scotland	0.68 ± 0.09	Camera trapping - Bayesian SECR models

Figure Captions

Figure 1 - Study area and the 1×1 km sampling grid; all detections are reported (near camera trapping sites +) assigning an individual symbol to each of the eleven European wildcats identified.

Figure 2 - Wildcats detected during the sampling period. a) Some frames and sequence of European wildcat videos in which the main morphological characteristics were shown; b) individual that presented intermediate morphological characteristics between the European wildcat (*Felis silvestris silvestris*) and the domestic cat (*Felis silvestris catus*).

Figure 3 - Spacing and number of detectors for the scenarios used in the simulations. a) 50 detectors spaced 800 meters apart; b) 38 detectors spaced 900 meters apart; c) 31 detectors spaced 1000 meters apart; d) 27 detectors spaced 1100 meters apart; e) 21 detectors spaced 1200 meters apart.

Figure 4 - Simulation results for non-pathological sampling designs. a) Number of individuals; b) Number of detections; c) Relative standard errors (RSE); d) Relative bias (RB).

Population density of European wildcats in a pre-alpine area (Northeast Italy) and an assessment of estimate robustness - Rebuttal letter

Dr. Krzysztof Schmidt comment

EDITOR: I have sent your revised paper to one of the reviewers that have previously evaluated your manuscript. Please, see the document uploaded on the Editorial Manager with all the comments provided by the reviewer. Overall, the reviewer found your paper greatly improved, but still requiring some edits. Please, make sure you follow the comments and suggestions carefully and revise the paper accordingly.

AUTHORS: Thank you for giving us the opportunity to revise the MS again. We accepted all the reviewer suggestions. The paper was also revised by an English native speaker, Crinan Jarrett from the University of Glasgow. Hereafter, you will find a point-by-point response to the major issues, while we simply fixed all the minor issues. We are very sorry for the one-day delay.

Reviewer major comments:

Reviewer #1: I believe that the authors have properly addressed the weak points in the simulation section, but I don't agree with the exclusion of a cat from the population density analysis because its status was not totally clear (i.e. putative hybrid). Overall, I feel that the readability and the English of this revised version are still not satisfactory and that some sentences were not located in the proper sections. I have provided comments for improving these flaws but given that I am not a native English speaker, I would recommend a thorough revision of the manuscript before resubmitting it. I hope the authors can expand their wildcat research in this study area.

Authors: We thank the reviewer for the precious comments and revisions, which helped improve the manuscript. We carried out again the population density analysis and simulations including the cat considered as putative hybrid. The paper was also revised by an English native speaker Crinan Jarrett from the University of Glasgow.

Reviewer minor issues

Authors: We accepted or fixed all the corrections/comments. Please refer to the new MS version for your evaluation.

Figure

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