Estimating Red Fox Density Using Non-Invasive Genetic Sampling and Spatial Capture-Recapture Modeling

Lars Lindsø (larslindso94@gmail.com)
Norwegian Institute for Nature Research: Norsk Institutt for Naturforskning https://orcid.org/0000-0002-5094-8583

Pierre Dupont
Norwegian University of Life Sciences Department of Ecology and Natural Resource Management: Norges miljo- og biovitenskapelige universitet Institutt for naturforvaltning

Lars Rød-Eriksen
Norwegian Institute for Nature Research: Norsk Institutt for Naturforskning

Ida Pernille Øystese Andersskog
Norwegian Institute for Nature Research: Norsk Institutt for Naturforskning

Kristine Roaldsnes Ulvund
Norwegian Institute for Nature Research: Norsk Institutt for Naturforskning

Øystein Flagstad
Norwegian Institute for Nature Research: Norsk Institutt for Naturforskning

Richard Bischof
Norwegian University of Life Sciences Department of Ecology and Natural Resource Management: Norges miljo- og biovitenskapelige universitet Institutt for naturforvaltning

Nina Elisabeth Eide
Norwegian Institute for Nature Research: Norsk Institutt for Naturforskning

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Abstract

Spatial capture-recapture modelling (SCR) is a powerful tool for estimating densities, population size and space use of elusive animals. Here, we applied SCR modeling to non-invasive genetic sampling (NGS) data to estimate red fox (*Vulpes vulpes)* densities in two areas of boreal forest in central (2016 - 2018) and southern Norway (2017 - 2018). Estimated densities were overall lower in the northern study area (mean = 0.04 foxes per km^2 [95%CI: 0.02-0.09] in 2016, 0.09 [0.05-0.18] in 2017 and 0.07 [0.04-0.13] in 2018) compared to the southern study area (0.16 [0.09-0.26] in 2017 and 0.10 [0.07-0.16] in 2018). We found a positive effect of forest cover on density in the northern, but not the southern study area. The absence of an effect in the southern area may reflect a paucity of evidence caused by low variation in forest cover, but could also be due to climatic differences (e.g., winter severity) between the two areas. Estimated mean home range size in the northern study area was 45 km^2 [34-60] for females and 88 km^2 [69-113] for males. Mean home range sizes were smaller in the southern study area (26 km^2 [16-42] for females and 56 km^2 [35-91] for males). In both study areas, detection probability was session-dependent and affected by sampling effort. This study highlights how SCR modeling in combination with NGS can be used to efficiently monitor red fox populations, and simultaneously incorporate ecological factors and estimate their effects on population density and space-use.

Introduction

Reliable information on animal population status, including population size and density, is crucial for wildlife research and management (Kämmerle et al. 2018). However, estimating population size and density is challenging. This is especially true for predators, because they often occur at low densities, are elusive and inhabit areas that may also be difficult to survey due to inaccessibility or rough terrain (Kery et al. 2011). Predators are also often of management concern due to their conservation status or conflict potential with humans through direct threat, depredation of livestock, competition for game species (Estes 1996) or spreading disease (Moore et al. 2010a).

The red fox (*Vulpes vulpes*) is a highly adaptable and opportunistic mesopredator with a broad ecological niche and variable diet, including both wild and domestic vertebrates (Dell'Arte et al. 2007; Killengreen et al. 2011). It is the most widely distributed carnivore in the world, and is commonly found in a wide array of habitats. It is also considered invasive or overabundant across much of its geographic range (Larivière & Pasitschniak-Arts 1996). The species' ongoing geographic expansion is of management concern due to deleterious effects on populations of other species, including competition with intraguild species like the arctic fox (*Vulpes lagopus*; Frafjord et al. 1989) and predation on prey species, including threatened species, like the lesser white-fronted goose (*Anser erythropus*; Aarvak et al. 2017) and game species, like forest birds (Doherty et al. 2016; Jahren 2017; Skrede 2016; Smedshaug et al. 1999). The red fox is also a vector of zoonotic diseases that can potentially pose risks for domestic animals and humans (Hodžić et al. 2016; Víchová et al. 2018, Laurimaa et al. 2016). Despite the importance of the red fox for wildlife management, few practical methods are available for estimating population size and densities, required

to evaluate effects of management actions (Wegge et al. 2019). Because direct observation of the red fox is difficult (Vine et al. 2009), methods used to monitor red fox populations have mainly been based on indirect measures, including culling indices (Smedshaug et al. 1999), snow tracking (Wegge and Rolstad 2011), fecal counts (Cavallini 1994; Webbon et al. 2004), mapping of active dens (Lindström 1989; 1994) and camera trap visits (Hamel et al. 2013; Henden et al. 2014). These methods assume that the measured indices are directly proportional to the population parameter of interest, be it population size or density. This relationship is, however, often unknown and thus the reliability and appropriateness of these methods are difficult to evaluate (O'Connell et al. 2010; Sollmann et al. 2013).

An alternative approach is capture-recapture (CR) methodology. CR methods are considered standard methodology in estimating animal population parameters (Silvy 2012), and use multiple captures of the same individual, identified by natural or artificial means, to make extended inferences at the population level. Two main advantages of CR methods are the ability to 1) account for imperfect detection, i.e., the fact that not all animals are detected, and 2) estimate variation in detection probability (Amstrup et al. 2010; Royle & Young 2008). A limitation of conventional CR methods, however, is the difficulty to estimate population density due to movements of animals into and out of the study area (Royle & Young 2008; Royle et al. 2018).

Unlike conventional CR methods, spatial capture-recapture (SCR) incorporates a spatially explicit component in the model that allows to account for the spatial heterogeneity in detection probability of individuals and, doing so, estimate density (Royle et al. 2013). In addition, SCR models allow for the incorporation of ecological factors such as sex or habitat characteristics, and estimate effects of these on population density and animal space-use. SCR is also well-suited for use in combination with non-invasive sampling methods, such as camera trapping and non-invasive genetic sampling (NGS) data (Mumma et al. 2015; Royle et al. 2013). NGS in combination with SCR methods has recently become a popular tool to monitor wide ranging carnivores at large scales (Bischof et al. 2020). Recent studies also support use of these methods to monitor mesopredators when applied at spatially appropriate scales (Morin et al. 2016; Wegge et al. 2019).

The goal of the present study is to assess the combination of non-invasive genetic sampling with spatial capture-recapture for estimating red fox density and explore the role of individual and spatial variables on density, space use, and detectability. Over a period of three years, we use data from two different study areas in Norway with different habitat and climate characteristics.

Materials And Methods

Study areas

The first study area (“Lierne”) was established in Lierne, Trøndelag in central Norway (64.353° N, 13.659° E; (Fig. 1A), where a pilot study was conducted in 2016. It consists of an undulating terrain between 500 and 950 m a.s.l. with mixed forests and protruding unforested crests, and a mean forest cover of 50 %.

Norway spruce (*Picea abies*) dominates the forests with interspersed Birch (*Betula spp.*), and Scots pine (*Pinus sylvestris*) (Moen 1998). Parts of the study area are subjected to commercial clear-cut forestry, and small settlements are scattered along the main road going through the study area. Parts of the region are used by semi-domestic reindeer (*Rangiferus tarandus*) for perennial pastures in addition to moose (*Alces alces*) and roe deer (*Capreolus capreolus*), and a diverse scavenger community is prevalent in both forested and alpine areas (Gomo et al. 2017; 2020).

The second study area ("Skrim") was established in 2017 in an area near Skrim, Viken in southern Norway (59.391° N, 9.590° E; Fig. 1B). This study area is located between 400 and 675 m a.s.l. and is comparable to the study area in Lierne in terms of species composition (Østbye 1989) and forestry practice (Moen 1998), but with denser forest cover (85 %), rougher topography, and no unforested crests. Human occupancy along the main roads through each study area is similar in both study areas, but the human population in adjacent settlements is much higher in Skrim. This includes a city (Skien) of 55 000 inhabitants, whereas the population of the entire municipality of Lierne is 1355 inhabitants (Statistics Norway 2020). Both study areas are 15 x 15 km (225 km^2; Fig. 1).

Data collection

Scats, urine, and hair from red fox were collected during February and March in 2016, 2017 and 2018 in Lierne, and in 2017 and 2018 in Skrim. The study areas were divided into regular 5 x 5 km grids. Sampling was done by local hunters and was primarily focused along snow covered dirt roads, snowmobile tracks, and skiing tracks. Urine samples were collected by placing spruce sticks for foxes to urinate on at an interval of approximately 500 meters along sampled roads and tracks. The same tracks were sampled at least twice for each study area each year. Scat, urine, and hair samples were handled with gloves and plastic cutlery to avoid contamination of DNA. The samples were placed in plastic vials containing silica gel or urine preservative fluid and paper envelopes, respectively, for preservation of DNA and storage for later analysis. All samples were dated and corresponding UTM coordinates were recorded with a handheld GPS unit.

DNA extraction, amplification and genotyping

The genetic analyses were undertaken at the Norwegian Institute for Nature Research (NINA) in Trondheim, Norway. DNA was extracted from 314 scat, 448 urine and 23 hair samples (Appendix A) using the FastDNA™ Spin Kit for Soil, the Norgen Biotek Urine DNA Isolation Kit (Slurry Format) and the Maxwell® 16 Tissue DNA Purification Kit, respectively, following the manufacturer's protocols. To confirm red fox samples, two PCR runs followed by capillary electrophoresis were performed for each sample using the species identification method described by Dalén et al. (2004). Samples from other species than red fox were excluded from further analysis. All confirmed red fox samples were genotyped with 14 microsatellite markers, including a marker for sex determination (Moore et al. 2010b). To account for genotyping errors in low-quality samples (Appendix D; E), three replicates per sample and marker were applied.

Consensus genotypes were assigned for each sample based on consistency across all three replicates for homozygote markers and at least two for heterozygotes. This procedure minimizes the risk of genotyping errors caused by allelic dropout and false alleles (Taberlet et al. 1996). To identify reliable genotypes, we assigned each sample a quality index (QI), calculated as the proportion of consistent gene scores across all three replicates, (Miquel et al. 2006). Samples with a mean QI of 0.70 or above were retained for subsequent individual identification. Finally, we assigned identities using Allelematch, an R package for identifying unique multilocus genotypes where genotyping error and missing data may be present (Galpern et al. 2012), in R version 3.6.0 (R core team 2019).

Spatial capture-recapture

General description

We estimated red fox densities for each study area and year using spatial capture-recapture (SCR) models. SCR models are hierarchical models composed of a submodel for the distribution of individuals in space, i.e., density (D), and a submodel for the detection of these same individuals, conditional on their location. SCR models assume that animals move within a certain range around a central point referred to as the activity center (AC). Density is modelled as the distribution of ACs over an area referred to as the state-space (Royle et al. 2013). Density may be modeled as a function of spatially explicit covariates (Borchers & Efford 2008). SCR models usually assume that the detection probability of an individual declines as the distance to its AC increases. The most common detection model is the half-normal function, which has two parameters. The scale parameter (σ; Royle et al. 2013) describes how fast the detection probability decreases with distance, and the baseline detection probability (p0) describes the probability to detect an individual at the exact location of its AC. Both the scale parameter and the baseline detection probability can be related to different individual or spatial covariates to account for potential heterogeneity in detection. The detection model also implies a model of space use that is closely linked with home range size through 1) movement of an individual about its home range and 2) detection being proportional to space use in the vicinity of a detector. We can thus use SCR models with a half-normal detection function to derive home-range size (i.e., the circular area encompassed by the 95% vertex of the utilization distribution) directly from the scale parameter σ using the chi^2-distribution with two degrees of freedom (Royle et al. 2013).

State-space, detectors and SCR data

Models were run separately for each study area and therefore the state-space and potential detection locations, i.e., detectors, were also study area-specific. The state-space for each study area was defined as a grid of 500m cells covering the area searched for DNA samples surrounded by a buffer of 5.4 km. The buffer width was based on the root pooled spatial variance (RPSV), a measure of the dispersion of detection locations of individual foxes, pooled over individuals and was defined as the highest RPSV across all sessions multiplied by three. Detectors were defined as the centers of 500 x 500 m grid cells covering each 25 x 25 km study area (N = 900; Appendix. F; G). Only samples found within the spatial bounds of the study areas (Fig. 1) for which coordinates, species, sex and individual ID were available

were considered a detection and assigned to the nearest detector. SCR datasets for each year and study area were then built as the number of individual detections at each detector.

Model implementation and selection

To test whether red fox density was correlated with available forest habitat, as suggested by previously reported habitat preferences of the red fox (Cagnacci et al. 2004; Svendsen 2016; Van Etten et al. 2007), we included an effect of forest cover on density. Proportion of forest cover for each state-space grid cell was extracted based on maps at scales between 1:25 000 to 1:100 000 (Norwegian Mapping Authority 2020) and converted from shape-format to a 500 m resolution raster in QGIS (Appendix F; G; QGIS development Team 2018). Based on the assumption that foxes select territories based on characteristics of the habitat at a scale larger than the area covered by a 500 x 500 m grid cell, we used the average forest cover within a 1000 m radius for each state-space grid cell.

To account for variation in detectability, we considered detector-specific covariates: search effort and road length. Search effort was defined as the total length of registered GPS search tracks within each detector grid cell and was included as a predictor on p0. Similarly, total length of roads within each detector grid cell, also based on maps at scales between 1:25 000 to 1:100 000 (Norwegian Mapping Authority 2020), was included as a predictor for p0. This was based on evidence suggesting that mesopredators often travel along roads in winter to conserve energy (Crête & Larivière 2003) and are therefore more likely to be detected close to roads. Both detector-specific predictor variables were standardized.

We included sex as a covariate on both p0 and σ as we wanted to test for sexual dimorphism in space-use for the red fox, as some studies report home range size of the red fox to differ between sexes (Drygala & Zoller 2013), while other studies report no significant sex differences (Svendsen 2016; Walton et al. 2017).

We constructed 16 candidate models based on all combinations of forest cover effect on density and road length, year, and sex effects on detection probability. All candidate models were extensions of a baseline model that contained predictors common to all candidate models. The baseline model included year-specific densities, search effort effect on p0 and sex effect on σ. Density was predicted to be session-dependent as density likely varies from year to year due to temporal variation in resource availability, recruitment, mortality, immigration and emigration, and because a main goal of the study is to report year-specific densities in each study area. Detection was predicted to be dependent on search effort, as detection probability is expected to be higher in areas that have been searched more. Lastly, space use was predicted to be sex-specific, as we expect males to have bigger home range sizes compared to females (Drygala & Zoller 2013). The set of candidate models included all combinations of forest cover on density and road length-, year-, and sex-dependent detection probability in addition to effects already incorporated in the baseline model.

All 16 models were run as multi-session sex-structured spatial capture-recapture models using the package oSCR (Sutherland et al. 2019) in R version 3.6.0 (R core team 2019). The sex-structured implementation allowed us to estimate sex ratio (ψ) and allowed use of data from different sessions, in this case years, in a single statistical model. This increases reliability and provides the opportunity to analyze effects on different parameters either jointly across sessions, or independently (Sutherland et al. 2019). Fitted models were subjected to post-processing and model selection fusing functionality provided in the oSCR package. Model selection was performed using the Akaike Information Criterion (AIC).

Results

NGS samples

Out of 502 total samples from Lierne, 383 were confirmed as red fox, of which 275 samples were successfully assigned reliable genotypes and individual IDs (for a breakdown by sample type, see Appendix A). Successfully genotyped samples originated from 98 different individuals. The mean number of samples per individual was 2.23 [95%CI 1.55–2.91]) in 2016, 2.57 [1.82–3.32]) in 2017 and 4.57 [3.02–6.01] in 2018 (Table 2). Out of 283 total samples from Skrim, 223 were confirmed as red fox, of which 103 were successfully assigned reliable genotypes and individual IDs. Successfully genotyped samples originated from 39 different individuals. The mean number of samples per individual was 1.72 [1.31–2.13] in 2017 and 2.40 [1.79–3.01] in 2018 (Table 1).

Table 1
Red fox non-invasive genetic sampling data per study area and year.

	Total no. of DNA samples	No. of red fox samples	No. of genotyped samples	No. of identified individuals	No. of identified females/males	Mean no. of samples per individual
Lierne 2016	160	76 (48%)	58 (36%)	26	19 / 7	2.23
Lierne 2017	184	155 (84%)	95 (51%)	37	20 / 16	2.57
Lierne 2018	158	152 (98%)	122 (77%)	27	12 / 15	4.52
Skrim 2017	150	102 (68%)	43 (29%)	25	11 / 14	1.72
Skrim 2018	133	121 (91%)	60 (45%)	25	12 / 13	2.40

Table 2
AIC-based model selection of red fox SCR models for Lierne (ΔAIC: AIC difference between each model and the lowest AIC; w_i: AIC weight).

Density (*D*)	Detection (*p0*)	Log-likelihood	No. of parameters	AIC	ΔAIC	w_i
session + forest	effort + session	881.11	13	1788.21	0.00	0.1933
session + forest	effort + session + road	880.12	14	1788.25	0.03	0.1903
session + forest	effort + session + sex	880.42	14	1788.84	0.63	0.1413
session + forest	effort + session + road + sex	879.51	15	1789.02	0.81	0.1289
session	effort + session + road	881.53	13	1789.06	0.85	0.1265
session	effort + session + road + sex	880.86	14	1789.72	1.51	0.0909
session	effort + session	883.18	12	1790.36	2.15	0.0660
session	effort + session + sex	882.36	13	1790.73	2.51	0.0550
session + forest	effort + road	886.70	12	1797.41	9.19	0.0019
session	effort + road	887.99	11	1797.97	9.76	0.0015
session + forest	effort + road + sex	886.15	13	1798.30	10.08	0.0013
session + forest	effort	888.47	11	1798.94	10.73	0.0009
session	effort + road + sex	887.47	12	1798.95	10.73	0.0009
session + forest	effort + sex	887.80	12	1799.60	11.38	0.0007
session	effort	890.54	10	1801.09	12.87	0.0003
session	effort + sex	889.89	11	1801.79	13.58	0.0002

Model selection

Of the 16 candidate models fitted for Lierne, the top model included forest cover as a predictor for density and year-specific baseline detection probabilities, in addition to the baseline predictors. Several models were very close with the top five models having a ΔAIC less than 1.0 (Table 2). For Skrim, the top model did not include an effect of forest cover on density, and baseline detection was influenced by year, road

length and fox sex, in addition to the baseline predictors. Models were also close in Skrim, with the top three models having a ΔAIC less than 1.0 (Table 3).

Table 3
AIC-based model selection of red fox SCR models for Skrim (ΔAIC: AIC difference between each model and the lowest AIC; w_i: AIC weight).

Density (D)	Detection (p0)	Log-likelihood	No. of parameters	AIC	ΔAIC	w_i
session	effort + session + road + sex	369.80	11	761.60	0.00	0.2041
session	effort + session + road	371.04	10	762.09	0.48	0.1602
session	effort + session + sex	371.24	10	762.47	0.87	0.1321
session + forest	effort + session + road + sex	369.39	12	762.79	1.18	0.1130
session	effort + session	372.43	9	762.86	1.26	0.1086
session + forest	effort + session + road	370.54	11	763.07	1.47	0.0980
session + forest	effort + session + sex	370.81	11	763.61	2.01	0.0746
session + forest	effort + session	371.90	10	763.80	2.20	0.0679
session	effort + road + sex	373.78	10	767.56	5.96	0.0104
session	effort + sex	375.22	9	768.44	6.84	0.0067
session	effort + road	375.34	9	768.67	7.07	0.0060
session + forest	effort + road + sex	373.42	11	768.84	7.24	0.0055
session	effort	376.80	8	769.60	7.99	0.0037
session + forest	effort + sex	374.85	10	769.70	8.10	0.0036
session + forest	effort + road	374.88	10	769.76	8.15	0.0035
session + forest	effort	376.33	9	770.65	9.05	0.0022

Estimated population size and density

Estimated red fox densities in Lierne were 0.04 [0.02–0.09] foxes per km^2 in 2016, 0.09 [05-0.18] in 2017 and 0.07 [0.04–0.13] in 2018. Furthermore, density was predicted to increase with forest cover (β_{forest} =

2.83 [0.02–5.63]; Fig. 2; Appendix H). Estimated population size within the original 225 km^2 study area in Lierne (Fig. 1A) was 12 [6–23] in 2016, 26 [15–46] in 2017 and 19 [11–34] in 2018.

Estimated red fox densities in Skrim were 0.16 [0.09–0.26] and 0.10 [0.07–0.16] foxes per km^2 in 2017 and 0.10 2018 respectively (Fig. 3), which corresponded to population sizes of 36 [20–59] and 23 [16–36] within the 225 km^2 study area in 2017 and 2018 respectively (Fig. 1B). Estimated sex ratios in both study areas were variable, but never significantly different from 0.5 (Appendix H; I). Mean densities for each study area per year are shown in Fig. 4.

Detection and space use

In Lierne, baseline detection probability increased with search effort (β_{search} = 0.76 [0.60–0.93]; Fig. 4; Appendix H). In Skrim, baseline detection probability increased with both search effort (β_{search} = 0.33 [0.12–0.54]) and road length (β_{road} = 0.2 [0.03–0.43]). In addition, detection probability was insignificantly lower for male than for female foxes in Skrim (β_{sex} = -0.76 [-1.71–0.19]; Fig. 5; Appendix I).

In Lierne, σ estimates were 1.55 [1.35–1.78] km for females and 2.17[1.91–2.46] km for males (Fig. 4), which corresponded to home range sizes of 45 [34–60] and 88 [69–113] km^2 for females and males respectively. In Skrim, σ estimates were 1.17[0.91–1.49] km for females and 1.73 [1.36–2.19] km for males (Fig. 5), corresponding to home range sizes of 26 [16–42] km^2 and 56 [35–91] km^2 respectively.

Discussion

Despite being the most widespread carnivore species globally, there is a paucity of detailed information about red fox population densities and their determinants. Using non-invasive genetic sampling and spatial capture-recapture analysis we mapped the density of red foxes in two boreal forest landscapes in Norway over three years. Our study revealed that a combination of spatial and individual factors influence density, space use, and detection probability.

We estimated higher red fox densities in the southern study area (Skrim) compared to the forest in central Norway (Lierne). Higher altitude, latitude, and a more continental climate, and lower winter temperatures make Lierne less productive than Skrim (Moen 1998). Reduced resource availability due to winter severity has been reported to be a limiting factor for red fox populations, where densities decrease with declining winter temperatures (Bartoń & Zalewski 2007). The difference in estimated red fox densities between the study areas may thus partially be attributed to difference in productivity and winter severity as limiting factors on density. Human land use and anthropogenic subsidies have been suggested to be important drivers of red fox density. Forest landscapes with high human settlement density are associated with higher red fox abundances, potentially driven by an increased food availability of anthropogenic origin, and thus increased scavenging opportunities (Jahren et al 2020; Rød-Eriksen et al. 2020). Both human activity in general and anthropogenic food sources are presumably higher in Skrim due to a larger human population as well as several clusters of cabins in adjacent areas compared to Lierne.

The two study areas differed not only in terms of their red fox densities, but also in terms of its predictors. Large-scale drivers of red fox density may differ according to variations in vegetation and climate. We detected a significant positive effect of forest cover on red fox density in Lierne but not in Skrim. Boreal forests are important habitats for several prey species of red fox in boreal forests including voles, shrews and forest birds (Lundstadsveen 2010). Forests may also provide important refuges in winter in contrast to more exposed alpine areas. Winters in Skrim are less severe and the link between food sources and forest cover less pronounced, possibly an explanation for a lack of an effect of forest cover on density in this study area. Alternatively, the lack of an effect of forest cover in Skrim may simply also reflect a paucity of evidence, perhaps because variation in forest cover was very low, with less open unforested areas like bogs and impediment (Appendix G).

The SCR models allowed us to derive sex-specific home range sizes. Home-range size estimates for both study areas were comparable to estimates from two recent GPS telemetry studies of red fox in similar habitat in Scandinavia. Svendsen (2016) reported mean red fox home-range size of 61 km2 [95%CI 25–105] for the region of Østerdalen, Innlandet, and Walton et al. (2017) reported mean home range sizes of 52 km2 [95%CI 32–72] for the regions of Kolmården, Grimsö, and Hedemora in Sweden, and Hedmark in Norway. However, the variation in reported home range estimates is significant in both studies. Walton et al. also reports home ranges up to four times larger in less productive and high elevation landscapes compared to more productive and low elevation landscapes (2017). We found a similar pattern with smaller home ranges in the more productive-lower elevation southern boreal forest (26 km^2 for females and 56 km^2 for males in Skrim), compared to Lierne's less productive-higher elevation northern boreal forest (45 km^2 for females and 88 km^2 for males). In contrast to studies by Svendsen (2016) and Walton (2017) that reported no differences in home range between males and females, our study found home range estimates of males to be approximately twice the size of females in both study areas. This may reflect variation in space use related to breeding status of females, as reproductive females have been reported to have smaller home ranges (Henry et al. 2005). Some females may have started retreating to natal dens towards the end of the sampling period (Walton & Mattisson 2021), which would affect their home range sizes. Furthermore, the DNA sampling was partly done during the foxes mating period (January-March), when male foxes likely roam around to cover several female home ranges (Cavallini 1996), which would contribute to the observed difference between males and females.

One important advantage of SCR is that it accounts for imperfect and variable detection of individuals. Though many count-based wildlife surveys assume complete detection of all individuals in a population, this assumption is almost always violated. When not accounted for, imperfect detection can lead to erroneous inferences about density and its drivers (Gu & Swihart 2004; Kellner & Swihart 2014). In addition, in most monitoring set-ups, detection probability differs amongst individuals in the population as a result of different exposure to detectors in relation to individual home range locations. SCR models use this inherent heterogeneity in detectability to estimate individual activity centers and space-use patterns (Royle et al. 2013). In in our study, variation in detection probability was also influenced by spatial predictors. Search effort had an expected positive effect on detection in both areas, and road

length tended to positively affect detection in Skrim (Appendix I). Lack of an effect of roads in Lierne may be due to insufficient evidence, as roads were fewer and covered less of the study area. However, the trend of an effect in Skrim could also be because roads and search transects coincided spatially (Appendix G). Detection probability also differed between years. Given that detection of individual animals depended on the genetic analysis of NGS-samples, this may reflect variation in genotyping success rates (Table 1), likely caused by year-to-year differences in weather and other environmental conditions that could affect the quality of samples.

A similar study by Wegge et al. (2019) produced SCR estimates of red fox densities that were comparable to estimates from more conventional CR methods. However, Wegge et al. report lower precision for their SCR density estimate (0.38 [95%CI 0.21–0.70]) compared to the present study, and argue that the smaller sampled area (50 km^2) is a main shortcoming of their study (2019). As carnivores usually occur at low densities, a study area approximately the size of one individual is likely too small to obtain good estimates of the population density (Maffei & Noss 2008).

Analysis of DNA from non-invasive sampling has become a viable method for individual identification of animals (Hausknecht et al. 2007; Woodruff et al. 2015). On average, 48 % of all samples collected in our study contained DNA of sufficient quality for individual identification. The proportion of successfully genotyped samples was noticeably higher in Lierne compared to Skrim (Table 1; Appendix A; D). This may be due to differences in climatic conditions that impact the degradation rate of DNA Evidence suggests that cold and dry conditions contribute to preservation of fecal DNA (Panasci et al. 2011; Piggott 2005; Woodruff et al. 2015). Thus, the difference in genotyping success may reflect environmental differences between the two study areas. Considering that the samples collected were of varying type and quality, the genotyping success rates reported here validate the NGS methods as viable for identifying individual foxes. Contributing factors could be the use of established species-specific markers in the genetic analyses, and a strict procedure for handling and storing samples. We especially recommend the use of gloves and single-use tools when handling samples in the field to avoid DNA contamination and using appropriate containers and preservatives for storing respective sample types.

The combination of SCR and NGS methods provides a solid framework not only to estimating red fox density, but also to identifying drivers thereof (e.g., productivity, snow depth, forest cover, influence of human activity). If applied at larger scales in different habitats, e.g., mosaics of forest and farmland or arctic and alpine areas, this approach has the potential to provide new insight into the relative importance of various drivers of red fox population dynamics.

The implementation of a red fox monitoring program based on NGS and SCR largely depends on appropriate sampling to ensure sufficient spatial redetections of individuals for each survey and successful DNA identification. Nevertheless, it is worth noting that SCR methods are currently being developed, and that several possibilities already exist to improve density estimates. Multiple data sources like recoveries of dead animals, for example, can be integrated to increase the precision of estimates (e.g.,

Bischof et al. 2020). Several methods were also recently proposed to incorporate detections of unidentified individuals, leading to improved estimation (Jimenez et al. 2019; Tourani et al. 2020).

Future analyses should also consider open-population SCR models. Yearly variation in density, as found in this study, could be due to annual variation in recruitment and mortality rates as well as culling rates. Indeed, open population SCR would allow for studying such population dynamics over time, including estimating mortality and recruitment rates, as well as immigration and emigration (Morin et al. 2016). This would also make better use of the available data, as information on individuals from one year can inform about individual states in other years (Milleret et al. 2020).

Declarations

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Author Contributions

NEE, LRE and ØF conceived the research idea. NEE, KRU, LRE and LKL coordinated the field work. IPØA and LKL performed the genetic analyses. LKL, PD and RB designed and performed the SCR analyses. LKL wrote the manuscript; and all authors contributed critically to the draft.

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Figures

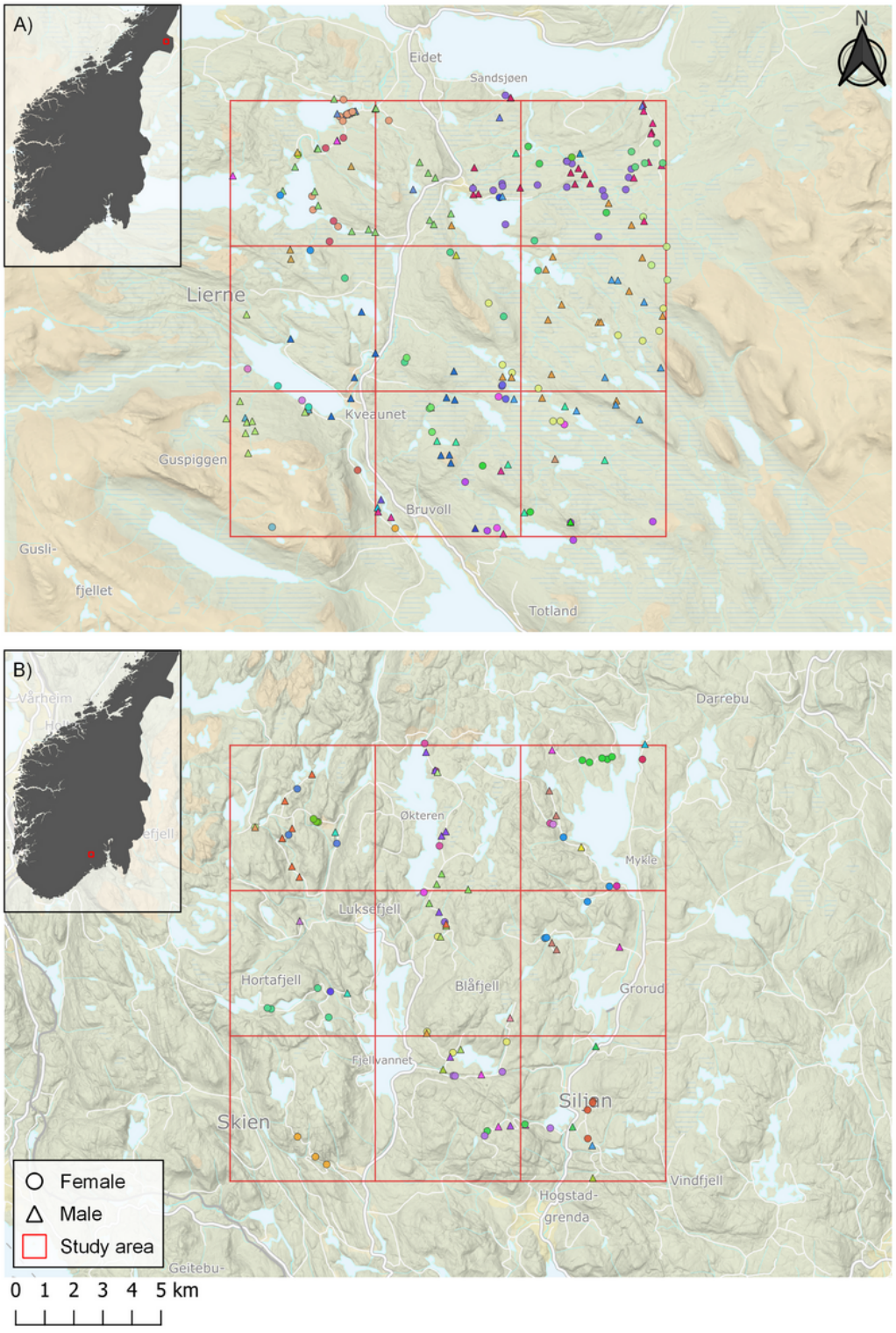

Figure 1

Map of the two 225 km2 study areas in A) Lierne in central Norway and B) Skrim in southern Norway. The study areas are shown with a 5 x 5 km grid with locations of all DNA samples included in the analysis, of which samples of the same color represent samples from the same individual. Inset panels show each study area's location in Norway. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square

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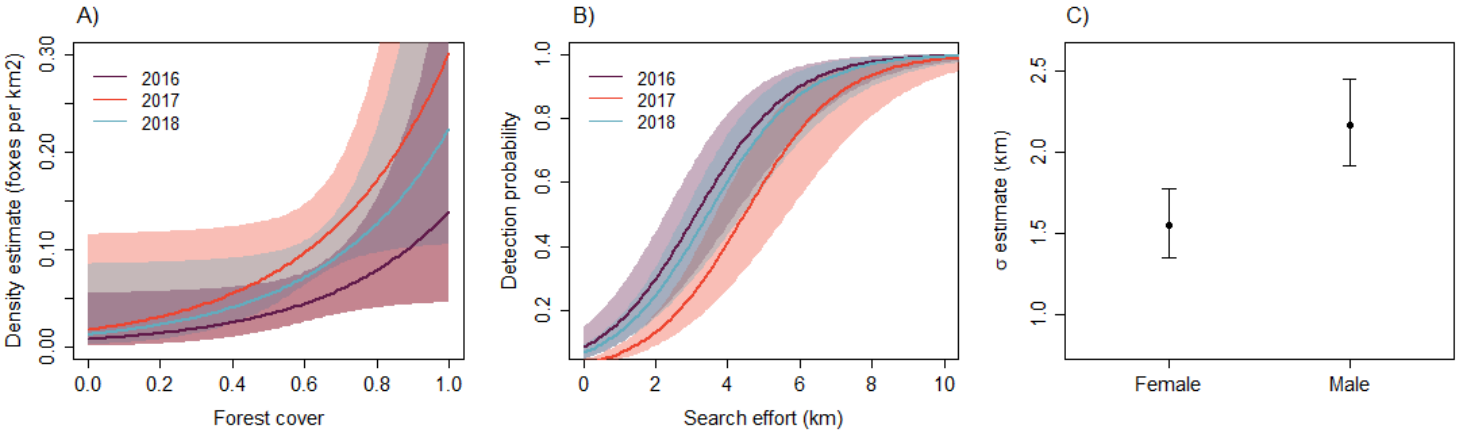

Figure 2

Predicted forest cover effect on density (A), predicted search effort effect on baseline detection probability (B), and sex difference in space-use (C) for the best supported model for Lierne. Colored lines represent the mean values and shaded polygons represent the 95% confidence intervals (A and B); dots represent the mean values and whiskers represent the 95% confidence interval (C).

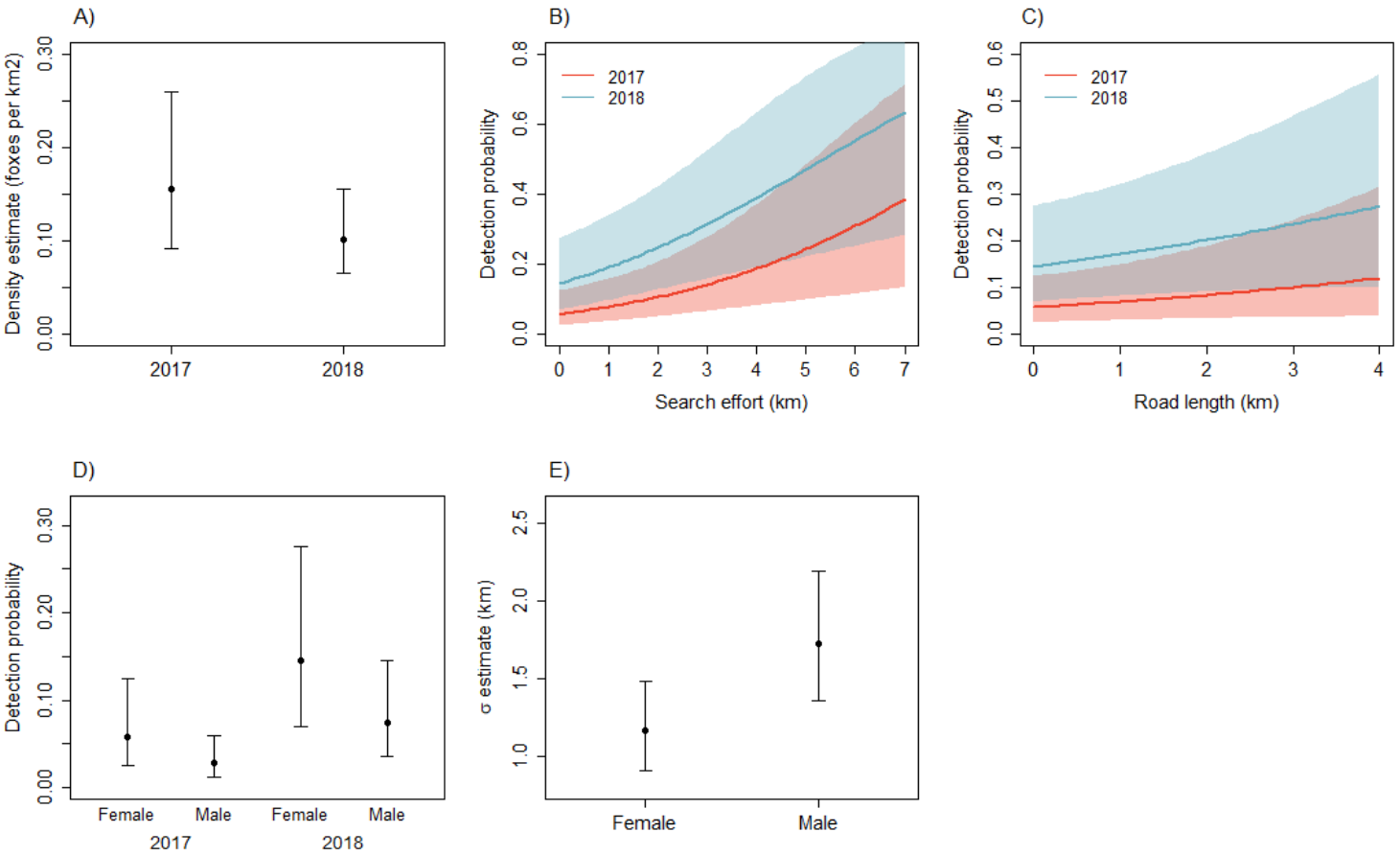

Figure 3

Estimated density per year (A), predicted effects of search effort (B; effect is shown for road length [scaled] = 0 and sex = male), road length (C; effect is shown for search effort [scaled] = 0 and sex = male) and session (D; effect is shown for road length [scaled] = 0 and search effort [scaled] = 0) on baseline detection probability, and sex difference in space-use (E) for the best supported model for Skrim. Colored lines represent the mean values and shaded polygons represent the 95% confidence intervals (B and C); dots represent the mean values and whiskers represent the 95% confidence interval (A, D, and E).

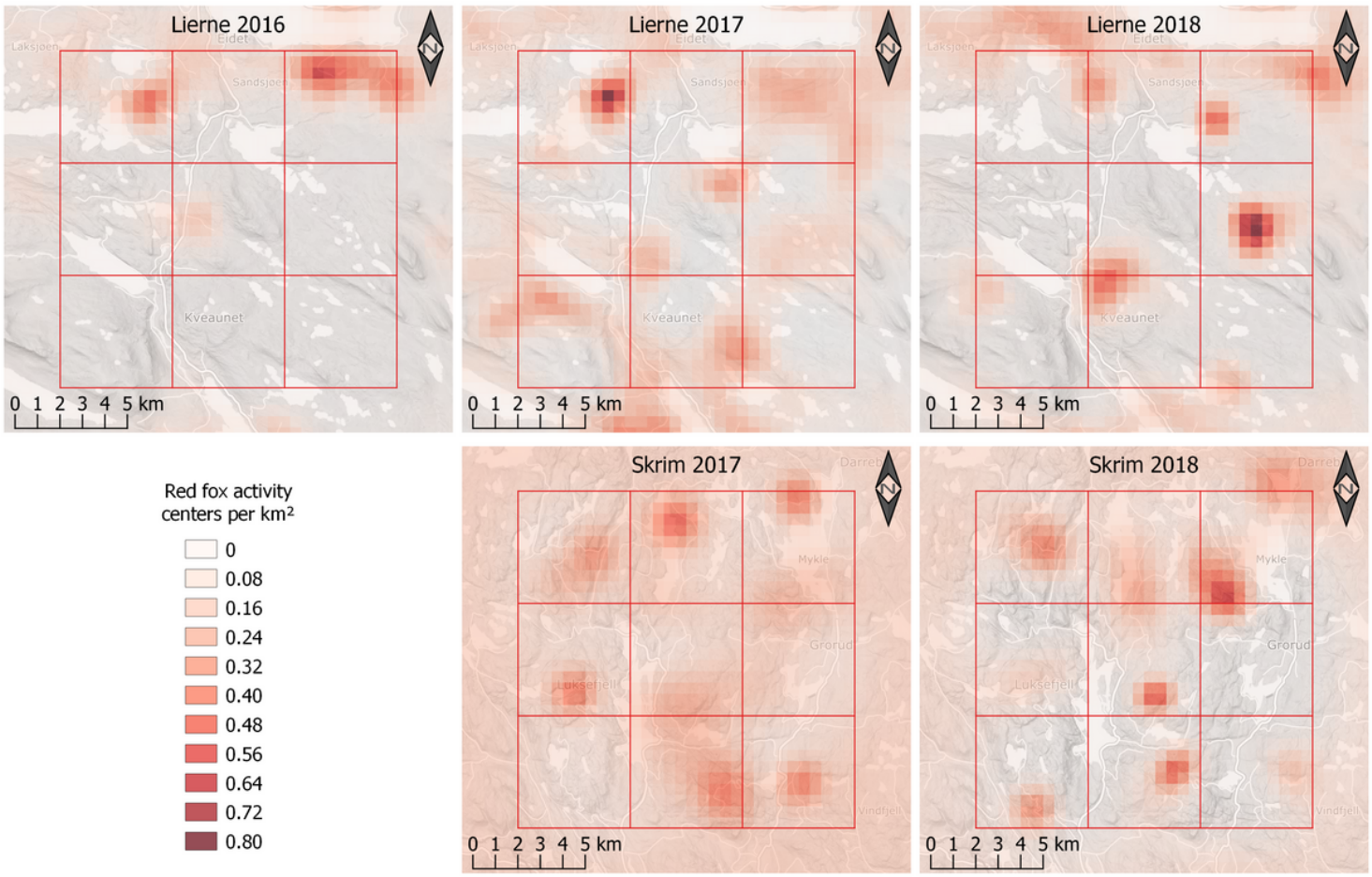

Figure 4

Mean red fox density in Lierne in 2016, 2017 and 2018, and Skrim in 2017 and 2018, derived from respective oSCR-models. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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