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Estimating densities of red foxes using non-invasive genetic sampling and spatial capture-recapture modeling

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Acknowledgments

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It has been very rewarding and educational to take part in all aspects of this project, from sample collection in the field to genetic analyses in the lab at NINA in Trondheim, and lastly doing the statistical analyses. I would like to especially thank Ida Pernille Øystese Andersskog at NINA for her guidance and patience with me in the genetics lab, where I analyzed around half of the samples in the study. Though I formally joined this project in the spring of 2019, I was first introduced to the project in early 2017 through my bachelor's degree program, where my class took part in the sampling process in Lierne. It has therefore been both a privilege and pleasure to see this project through to the end.

Abstract

Spatial capture-recapture modelling (SCR) is a powerful tool for estimating densities, population size and space use of elusive animals. However, SCR modeling has not been widely applied to ecologically important mesopredators like the red fox. Here I apply SCR modeling in combination with non-invasive genetic sampling (NGS) to estimate red fox (*Vulpes vulpes)* densities in two 225 km^2 areas of boreal forest in Lierne municipality in central Norway (2016-2018) and in the Skrim in southern Norway (2017-2018). DNA from scats, urine and hair was extracted, amplified and genotyped using 13 microsatellite markers. Of 785 samples collected, 378 (48 %) were successfully genotyped and given individual IDs. From these, 60 unique individuals were identified in Lierne and 38 in Skrim. I compared 16 candidate SCR models including spatial covariates on density and detection using AIC.

Estimated densities in Lierne were 0.04 foxes per km^2 (95%CI 0.02-0.09) in 2016, 0.09 (95%CI 0.05-0.18) in 2017 and 0.07 (95%CI 0.04-0.13) in 2018. In addition, density was predicted by a forest cover habitat covariate where density increased exponentially with forest cover (beta = 2.83; 95%CI 0.02-5.63). Estimated home range size in Lierne was for 45 km^2 (95%CI 34 - 60) for females and 88 km^2 (95%CI 69 – 113) for males. In Skrim, the top model did not include a habitat covariate on density. Estimated densities were 0.16 foxes per km^2 (95%CI 0.09-0.26) in 2017 and 0.10 (95%CI 0.07-0.16) in 2018. Estimated home range size in Skrim was 26 km^2 (95%CI 16 - 42) for females and 56 km^2 (95%CI 35 - 91) for males.

Results reported here validate SCR modeling in combination with NGS as a reliable method for monitoring red fox populations. Methods applied can be developed further to include different types of data for more precise estimates, including DNA from recoveries of dead foxes and partially identified individuals.

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1.0 Introduction

Reliable information on animal population status, including population size and density, is crucial for wildlife research and management (Kämmerle et al. 2018). To study population dynamics over time and evaluate the effectiveness of management measures, implementing wildlife monitoring programs plays an essential role (Barea-Azcón et al. 2006). Monitoring is important at larger scales (Jones 2011), e.g. for national management of a species. It is also important at smaller scales, as management regimes often are geographically differentiated, and require spatially appropriate information for local decision-making (Kämmerle et al. 2018). However, estimating population size and density of animals with elusive behavior, particularly predators, is often challenging due to their low densities, are difficult to detect, and because they often inhabit areas that are of large spatial extent or are otherwise difficult to survey due to inaccessibility or rough terrain (Kery et al. 2011).

Predators are often of management concern due to their conservation status or because of their impact on prey species (Kämmerle et al. 2018), including livestock (Berger 2006). The red fox (*Vulpes vulpes*) is a highly adaptable and opportunistic generalist 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 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). Red foxes can take advantage of many different food sources, including edible waste left in the wake of human activities (Jahren et al 2020; Rød-Eriksen et al. 2020). The ability to exploit such niches can have detrimental effects on intraguild competitor species like the pine marten (*Martes martes*) in forest habitats or arctic foxes (*Vulpes lagopus*) in alpine areas (Frafjord et al. 1989; Smedshaug et al. 1999). Predation pressure from the red fox can also have deleterious effects on populations of prey species (Doherty et al. 2016), including important game species like forest birds (Jahren 2017; Skrede 2016), and threatened species like the lesser white-fronted goose (*Anser erythropus*; Aarvak et al. 2017). In areas where the structure and dynamics of the landscape and original habitat have been altered by human land use, modern agriculture and forestry, red fox activity concentration is often seen along edges of forest habitat islands, often called edge-effects (Storch et al. 2005; Svobodová et al. 2011; Kurki et al. 1998). This can amplify predator-prey dynamics and have detrimental effects on

populations of prey species through increased predation pressure (Andrén 1995). Bounties are often offered as incentive to cull local red fox populations and reduce conflict with both farmers and hunters (Wegge et al. 2019). However, despite red foxes being a common and important species for wildlife management, few practical methods are available for estimating population size and density, monitoring population dynamics over time, and thus also the ability to evaluate effects of management actions for the species (Wegge et al. 2019). Because direct observation of the red fox is difficult (Vine et al. 2009), methods used to monitor populations have mainly been based on indirect measurements of abundance, 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, known as relative abundance indices (RAIs), are often applied on species that are difficult to identify individually (O'Connell et al. 2010). They share the assumption 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 performance and applicability of the RAIs are difficult to evaluate (O'Connell et al. 2010; Sollmann et al. 2013).

An alternative to RAIs are various capture-recapture (CR) methods. CR methods are considered standard methodology in estimating animal population parameters (Silvy 2012), and involve using 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 present are detected, and 2) estimate variation in detection probability (Amstrup et al. 2010; Royle & Young 2008). A limitation of conventional CR methods, however, is their inability to estimate population density due to unpredictable movement of animals into and out of the area where sampling takes place. The space around the surveyed area in which animals move, called effective sampled area, is thus difficult to define (Royle & Young 2008; Royle et al. 2018). Various ad hoc methods have been developed to circumvent this problem, e.g. by buffering the study area to account for individuals with home ranges that extend beyond the sampled area (Jimenez et al. 2019; Karanth & Nichols 1998). Another deficiency of conventional CR methods is their inability to account for the fact that animals in the periphery of the sampled area are less likely to be detected than animals with their home range well within the sampled area (Fig. 1). This heterogeneity in detection probability of individuals is

known to result in negative bias of density estimates (Otis et al. 1978; Royle et al. 2018). Unlike conventional CR methods, spatial capture-recapture (SCR) incorporate a spatially explicit component in the model for estimating density. Based on the detection locations of individuals detected more than once, SCR models estimate each animal's activity center, from which heterogeneity in detection probability is inferred as a function of distance between the activity centers and where they are detected. The density of activity centers can then be estimated across a buffered area around the detector array, thus circumventing the problem of estimating the effective sampled area. Activity centers of undetected individuals can also be estimated using the spatial heterogeneity in detection probability and the spatial distribution of activity centers of detected individuals within the study area. In addition, SCR models allow for the incorporation of other ecological factors influencing the different model parameters, including sex and habitat covariates, and estimate effects of these. SCR can be used in combination with non-invasive sampling methods, including camera trapping and genetic sampling (Royle et al. 2013). Non-invasive genetic sampling (NGS) provides a reliable method for identifying individual animals (Mumma et al. 2015). NGS has recently become a prominent tool applied in combination with SCR methods to monitor wide ranging carnivores at large scales (e.g. Bischof et al. 2019). Recent studies also support application of these methods to monitor mesopredators when applied at spatially appropriate scales (Morin et al. 2016; Wegge et al. 2019).

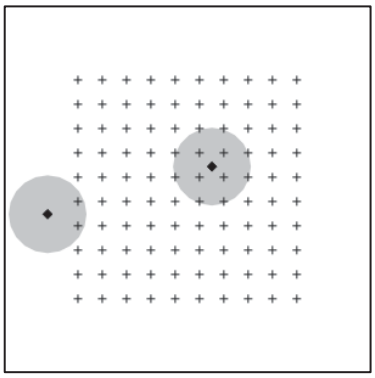

Figure 1: Spatial detector grid with home ranges of two individuals (gray circles) illustrating difference in exposure to the detector grid based on home range location.

This study is part of a larger project headed by the Norwegian Institute for Nature Research (NINA) that aims at investigating predator-prey dynamics and other ecological processes influencing the abundance and density of red fox and its prey species in Norway. A major goal of the project is to develop, evaluate and establish new methods based on non-invasive sampling for estimating abundance of red fox populations for science and management. The goal of my study is thus to assess the combination of non-invasive genetic sampling with spatial capture-recapture methods for estimating red fox densities based on their application in two different study areas in Norway over a period of three years. Moreover, I investigated the effects of different covariates on red fox density, detection and space use, including both spatial determinants on density and detection, and individual determinants on space use.

2.0 Methods

2.1 Study areas and data collection

2.1.1 Study areas

The first study area was established in Lierne, Trøndelag, where a pilot study was conducted in 2016 (Fig. 2). The study area was selected on the basis of being a relatively homogenous boreal forest habitat. It consists of a slightly 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*) dominate the forests with interspersed Birch (*Betula spp*.) and Scots pine (*Pinus sylvestris*). Parts of the study area are subjected to commercial clearcut forestry, and there are smaller settlements scattered along the main road going through the study area. Another study area was established in 2017 in an area near Skrim, bordering Viken and Agder (Fig. 2). 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 both forest community composition and forestry practice. It does however differ slightly due to having denser forest cover (85 %), rougher terrain topography-wise, and no unforested crests. Human occupancy along the main roads through each study area is similar, but the human population in adjacent settlements is much higher in Skrim, which includes Skien; a city of 55 000 inhabitants, whereas there are 1355 inhabitants in Lierne municipality (SSB 2020). Both study areas are 15 x 15 km (225 km^2; Fig. 2).

2.1.2 Data collection

Scats, urine and hair from red fox were sampled in the winter seasons of 2016, 2017 and 2018 in Lierne, and in 2017 and 2018 in Skrim. Sampling was done during February and March in both study areas. The study areas were divided into 5 x 5 km grids for ease of sampling coordination. Sampling was done by local hunters and was primarily focused along snow covered dirt roads, snowmobile tracks and skiing tracks. Urine samples were taken by placing spruce sticks for foxes to urinate on at an interval of approximately 500 meters along sampled roads and tracks. Sampling was done a minimum of two times in the same search track for each study area each year. Scat, urine and hair samples were handled with gloves and plastic cutlery to avoid contamination of DNA and put in glasses containing silica gel, urine preservative fluid and paper envelopes, respectively, for preservation of DNA and storage for later analysis. All samples were dated and positioned in UTM coordinates with a GPS, and all searches were logged in tracklogs for later analysis of detection probability in relation to search effort.

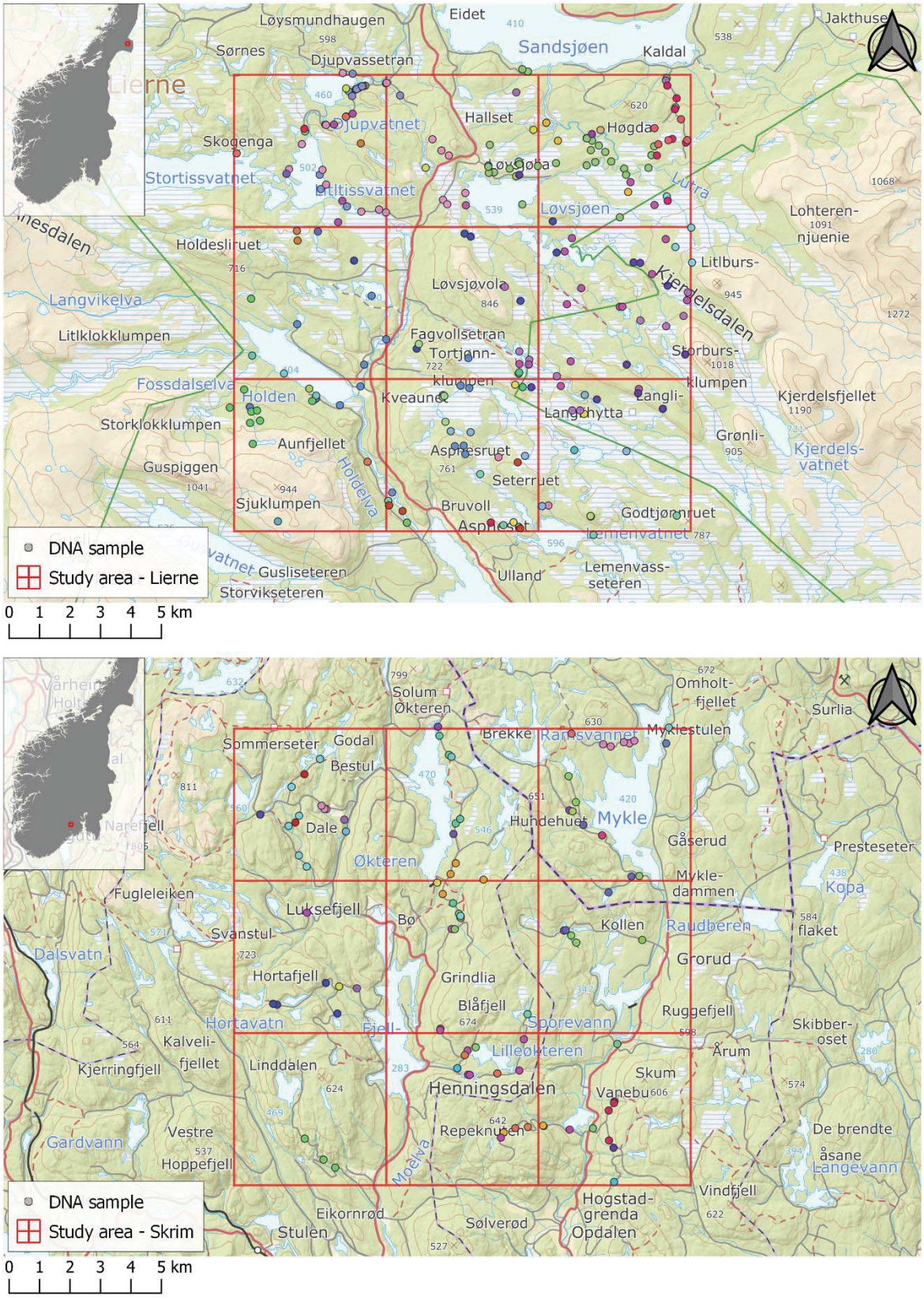

Figure 2: Map of the two 225 km^2 study areas Lierne (top) and Skrim (bottom). The study areas are shown with a 5 x 5 km grid with locations of all DNA samples included in the analysis, of which samples of the same color represent samples from the same individual. Inset panels show each study area's location in Norway.

2.2 DNA analyses

2.2.1 DNA Extraction

Laboratory analysis of the samples was done at the Norwegian Institute for Nature Research (NINA) in Trondheim, Norway. DNA was extracted from scat, urine and hair samples 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. All DNA extractions were done following the respective manufacturer's manuals.

2.2.2 DNA Amplification

All samples were first amplified in polymerase chain reactions (PCR) using 6 species specific microsatellite markers to confirm red fox samples. Two replicates were run for each sample, followed by capillary electrophoresis. Sequences from other species than red fox were excluded from further analysis.

To identify individual foxes, all samples of confirmed red foxes were genotyped using 13 microsatellite markers combined in two mixes (mix A: AHT133, C08.618, CPH2, FH2001, FH2054, FH2328, FH2457 and mix B: AHTh171, CPH11, CPH18, CPH7, CXX-468, FH2848, K9-Amelo, REN54P1; Table 1; Moore et al. 2010). Polymerase chain reactions (PCR) were replicated three times per sample. Each PCR was performed in 8,4 µL reactions composed of 4 µL of Qiagen Multiplex mix containing HotStarTaq DNA Polymerase, 1,6 µL of RNase-free H_2O, 0,8 of µL primer mix with adjusted primer set concentrations (Appendix B) and 2 µL of DNA template. The three last samples of each 96-well plate were controls; two positive and one negative. Quality checked red fox DNA from tissue samples were used as positive controls, while 1 µL of RNase-free H_2O was used as negative controls.

The PCR conditions for all tests were 95 °C for 15 min, 35 cycles of 95 °C for 30 sec, annealing at 57 °C for 90 sec, 72 °C for 60 sec, and a final extension of 60 °C for 30 min. The PCR reactions were carried out on a Veriti 69 Well Thermal Cycler (Applied Biosystems).

Table 1: Microsatellite markers used in the analysis, with respective multiplex panels, dye colors, allele number and size range (bp = base pairs). * = sex marker

Marker	*Mplex*	*Dye*	*Allele number*	*Allele range (bp)*
AHT133	MIX A	Red	9	85-111
C08.618	MIX A	Red	7	190-202
CPH2	MIX A	Yellow	8	93-111
FH2001	MIX A	Blue	4	132-150
FH2054	MIX A	Yellow	7	142-198
FH2328	MIX A	Green	9	131-167
FH2457	MIX A	Green	11	280-323
AHTh171	MIX B	Red	5	155-167
CPH11	MIX B	Blue	3	114-118
CPH18	MIX B	Blue	8	168-192
CPH7	MIX B	Yellow	4	154-176
CXX-468	MIX B	Green	7	82-94
FH2848	MIX B	Green	6	221-235
*K9-Amelo**	MIX B	Yellow		202, 216
REN54P11	MIX B	Red	9	189-219

2.2.3 Genotyping

All PCR products were mixed with GeneScan 500 LIZ (Applied Biosystems) size standard (0.16 µL) and Hi-Di formamide (7.84 µL), followed by capillary electrophoresis on a Hitachi 3500xL Genetic Analyser (Applied Biosystems) for genotyping using the POP-7™ Polymer as separation matrix. Results from the capillary electrophoreses were analysed in GeneMapper 5.0 (Applied Biosystems). All genotypes were assigned automatically by the software and subsequently checked manually. I determined consensus genotypes for each marker based on consistency between three replicates for homozygote markers and at least two for heterozygotes. This procedure minimizes the risk of genotyping errors caused by allelic dropout and polymerase errors in poor quality samples (Taberlet et al. 1996). Peak height was generally scored if it exceeded 500 relative fluorescent units (RFU), but peaks >200 RFU were scored if peak patterns were irrefutable and consistent between replicates. To identify reliable genotypes a quality index (QI), calculated as the proportion of consistent allele scores across all 3 replicates was assigned for each sample (Miquel et al. 2006). Samples with a mean QI of 0.70 or above were assigned consensus genotypes for subsequent individual identification. Finally, I 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).

2.3 Spatial capture-recapture modeling

2.3.1 SCR model

I estimated red fox densities for each study area and year using spatial capture-recapture (SCR) modeling, using the package oSCR (Sutherland et al. 2019) in R version 3.6.0 (R core team 2019). SCR models assume that animals move within a certain range from a central point referred to as its activity center. These activity centers are distributed over an area referred to as the state space (Royle et al. 2013). The density (D) of these activity centers may vary within the state space in relation to spatially explicit covariates, of which effects can be estimated (Borchers & Efford 2008). In addition, SCR models assume a half-normal parametric model $p(D) = p0\exp(-D^2/2\sigma^2)$ relating the detection probability of individual animals as a function of a scale parameter, here referred to as sigma (σ). The scale parameter controls how detection probability declines with distance from their activity center (Royle et al. 2013). Detection probability of an individual at the location of its activity center is referred to as p0. In count-based animal studies detection is often assumed to be complete and invariant. However, detecting all animals in a population is rarely feasible due to observer error, elusive animals or environmental variables affecting detection. When not accounted for, imperfect detection can lead to erroneous density estimation and biased relationships of related ecological covariates like habitat (Gu & Swihart 2004; Kellner & Swihart 2014). Because SCR models implement a parametric model relating detection probability to the distance from estimated activity centers in addition to the covariates on detection, imperfect and variable detection is estimated and accounted for (Royle et al. 2013).

The detection model also implies a model of space use through 1) movement of an individual fox about its home range, i.e. its utilization distribution, and 2) detection being conditional on space use in the vicinity of a detector. We can thus estimate home range size (e.g. using the 95% kernel density) directly from the scale parameter of the detection function σ (Royle et al. 2013). Furthermore, the implementation of SCR models in oSCR is that of a multi-session sex-structured spatial capture-recapture model, and as such estimates sex ratio (psi) and allows using data from different years or sessions 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).

2.3.2 Capture data, detector grid and state space buffer

I ran multi-session models for each study area separately, where input files for each model included a capture file, respective detector files for each year and a defined state space. The capture file included information about locations of all detections of identified individual foxes for each study area. Only samples found within the spatial bounds of the study areas (Fig. 1) for which coordinates, species, sex and individual ID were available were used in the analysis. The detector array was defined by the center of 500 m grid cells covering the spatial bounds of the study areas. The detector file included coordinates for each detector (N = 900), and a binary operation value, indicating whether each grid cell was searched or not. The state space was defined by creating a 5,4 km buffer around the respective study areas. The theory behind SCR models says that the buffer width of the state space has to be wide enough so that foxes at the edge of the state space effectively have zero chance of being detected inside the study area, where a buffer of $\geq 2\sigma$ is suggested for unbiased density estimates (Royle et al. 2013). This buffer was therefore based on the root pooled spatial variance (RPSV), a measure of the dispersion of the detection locations of individual foxes, pooled over individuals. As such, it represents a biased estimate of the scale parameter σ, ignoring the problem that movement of foxes is truncated by the spatial bounds of the detector grid (Efford 2020). The buffer value was calculated in R and was defined as highest RPSV for each study area multiplied by three.

2.3.3 Covariates

The capture input file included sex as an individual covariate. The detector file included detector-specific covariates on search effort and road length (Fig. 3; Fig. 4). Search effort was defined as total meters searched within each detector grid cell and was included as a predictor on p0. Similarly, total length of roads within each detector grid cell was included as a covariate on p0. This was based on evidence suggesting that mesopredators often travel along roads in winter for conserving energy (Crête & Larivière 2003) and are therefore more likely to be detected close to roads. Both detector specific covariates were standardized. To test whether red fox density was spatially 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), the state space included a forest cover covariate on density. Both forest cover and road length were based on N50 map data adapted for use with maps at scales between 1:25 000 to 1:100 000 (Kartverket 2020).

The forest cover layer was transformed to a 500 m resolution raster in QGIS (QGIS development Team 2018) and imported to R (R core team 2019). Going from an assumption that foxes likely select territories based on the habitat available at a larger scale than the area of 500 m grid cells, mean forest cover was extracted in a 1000 m radius around each raster pixel (Fig. 3; Fig. 4).

2.3.4 Model selection

Multi-session models of varying complexity were specified with covariates from the input files as predictors for the three main model parameters density (D), detection probability (p0), and space use (σ). A set of 16 candidate models were fit, after a baseline model was first defined. The baseline model included session-dependent density, effort-dependent detection probability and sex-dependent sigma. Density was assumed to be session-dependent as density is likely to vary 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 assumed to be dependent on search effort based on the method for data collection, as detection probability is expected to be higher in areas that have been searched more and vice versa. Lastly, space use was assumed to be sex specific, as daily distance traveled of red foxes have been shown to differ between sexes, where males travel longer distances than females (Servín et al. 1991). This relationship was assumed to be constant across years, thus session specificity was not tested for.

The set of candidate models included all combinations of forest cover on density and road length, session and sex dependent detection probability in addition to effects already incorporated in the baseline model. Using built-in post processing and model selection functions in the oSCR package, the fitted models were compared using Akaike Information Criterion (AIC).

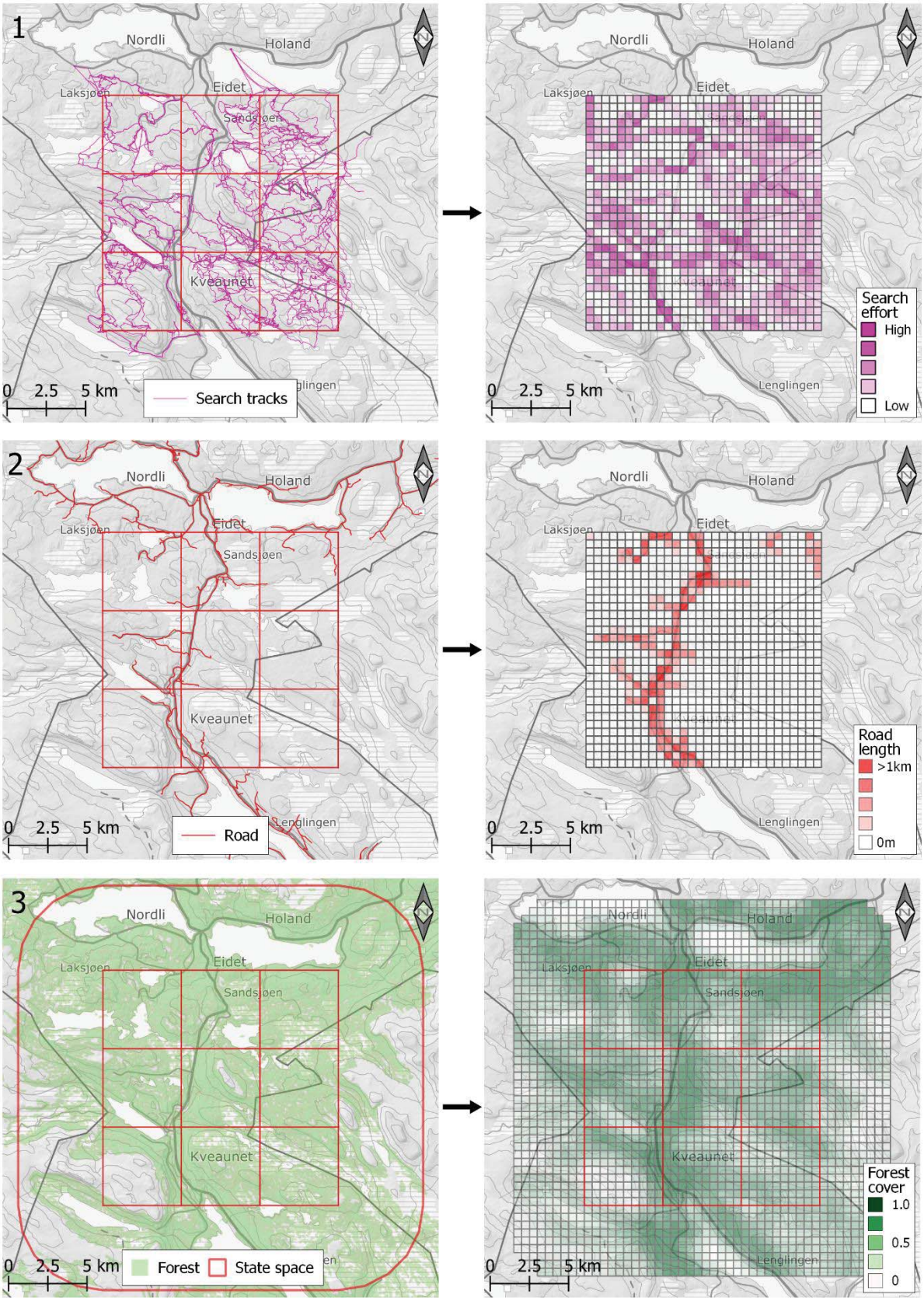

Figure 3: Visualization of spatial covariates on model parameters for Lierne (from top to bottom):
1. Search effort on detection per detector from tracklogs.
2. Road length on detection per detector from N50 data (Kartverket 2020).
3. Forest cover on density per state space cell from N50 data (Kartverket 2020).

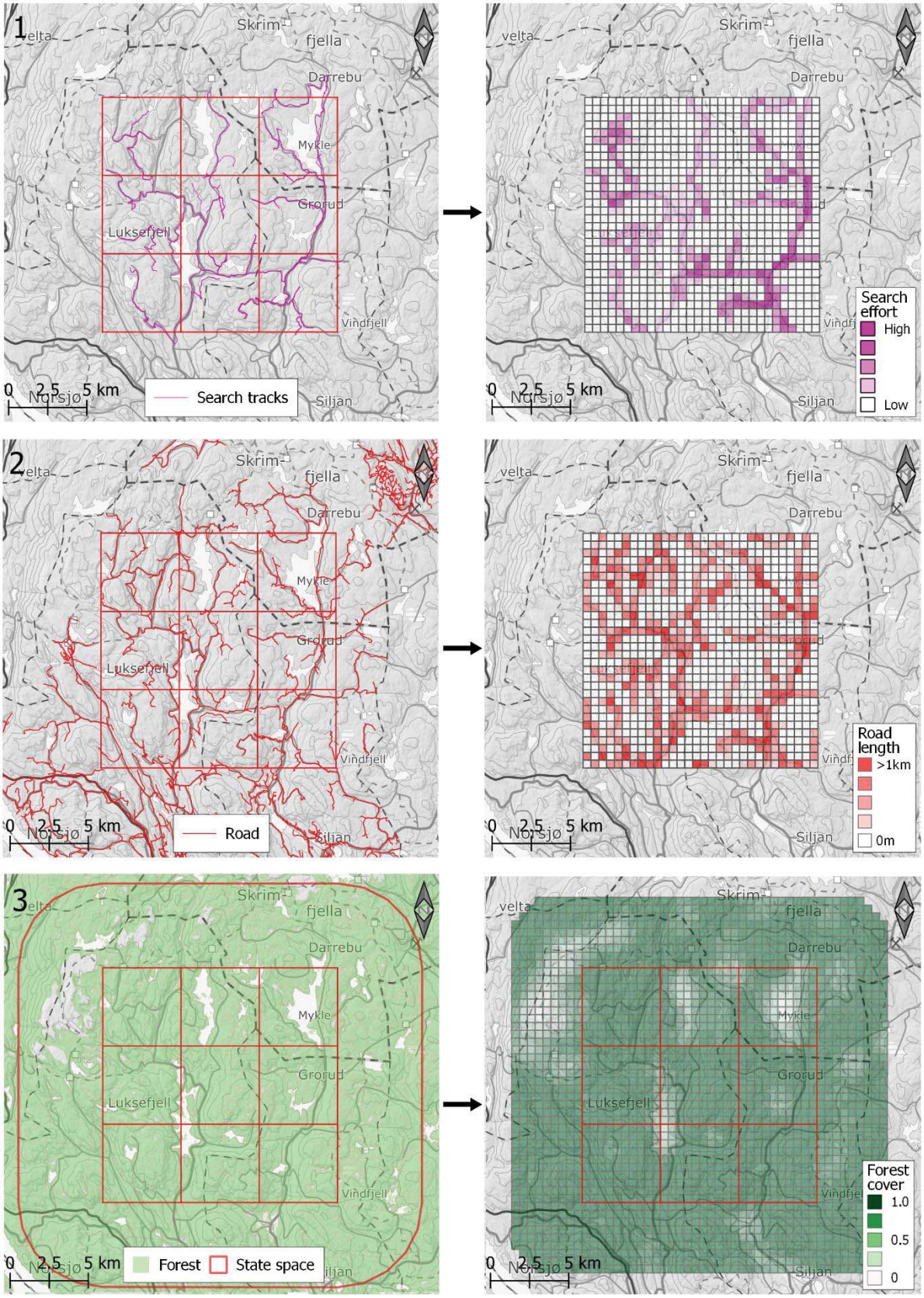

Figure 4: Visualization of spatial covariates on model parameters for Skrim (from top to bottom):
1. Search effort on detection per detector from tracklogs.
2. Road length on detection per detector from N50 data (Kartverket 2020).
3. Forest cover on density per state space cell from N50 data (Kartverket 2020).

3.0 Results

3.1 NGS samples

Out of 502 total samples in Lierne, 383 samples were confirmed as red fox, of which 275 samples were successfully assigned reliable genotypes and individual IDs. The successfully genotyped samples came from 98 different individuals. The mean number of samples per individual was 2.23 (SD 1.76) in 2016, 2.57 (SD 2.33) in 2017 and 4.57 (SD 3.97) in 2018 (Table 2). Out of 283 total samples in Skrim, 223 samples were confirmed to be red fox, of which 103 samples were successfully assigned reliable genotypes. Successfully genotyped samples originated from 39 different individuals. The mean number of samples per individual was 1.69 (SD 1.03) in 2017 and 2.40 (SD 1.55) in 2018 (Table 2).

Table 2: Summary of sampling data per study area and year, including total number of samples, number of confirmed red fox samples, number of successfully genotyped samples, number of identified individuals and mean number of samples per individual.

	Total no. of samples	No. of red fox samples	No. of genotyped samples	No. of identified individuals	Mean no. of samples per individual
Lierne 2016	160	76 (48%)	58 (36%)	26	2.23
Lierne 2017	184	155 (84%)	95 (51%)	37	2.57
Lierne 2018	158	152 (98%)	122 (77%)	27	4.52
Skrim 2017	150	102 (68%)	43 (29%)	26	1.69
Skrim 2018	133	121 (91%)	60 (45%)	25	2.40

3.2 Model selection

The top SCR model in the set of candidate models differed between study areas. For Lierne, the best model according to AIC included an effect of forest cover on density, and an effect of session on detection probability in addition to the effects already incorporated in the baseline model (Table 2) The best model according to AIC in Skrim did not include an effect of forest cover on density, but included an effect of both road length and sex on detection probability in addition to the effects already incorporated in the baseline model (Table 3).

Table 3: Compared red fox (Vulpes Vulpes) oSCR models in candidate set for Lierne using Akaike Information Criterion (AIC), including the parameters included in respective models on density (D) and detection (p0), log-likelihood, difference of AIC between each model and the lowest AIC (ΔAIC), and the weight of evidence per model (w_i).

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

Table 4: Compared red fox (Vulpes Vulpes) oSCR models in candidate set for Skrim using Akaike Information Criterion (AIC), including the parameters included in respective models on density (D) and detection (p0), log-likelihood, difference of AIC between each model and the lowest AIC (ΔAIC), and the weight of evidence per model (w_i).

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

3.3 Estimated population size and density

Estimated red fox densities in Lierne were 0.04 foxes per km^2 (95%CI 0.02-0.09) in 2016, 0.09 (95%CI 0.05-0.18) in 2017 and 0.07 (95%CI 0.04-0.13) in 2018. Furthermore, density was predicted by forest cover where higher densities were estimated in more forested areas with a beta coefficient of 2.83 (95%CI 0.02-5.63; Fig. 5; Appendix E). Estimated population size within the original 225 km^2 study area ion Lierne (Fig. 2) was 12 (95%CI 6 - 23) in 2016, 26 (95%CI 15 – 46) in 2017 and 19 (95%CI 11 - 34) in 2018. Estimated red fox densities in Skrim were 0.16 foxes per km^2 (95%CI 0.09-0.26) in 2017 and 0.10 foxes per km^2 (95%CI 0.07-0.16) in 2018 (Fig. 5). Estimated population size within the 225 km^2 study area (Fig. 2) was 36 (95%CI 20 - 59) in 2017 and 23 (95%CI 16 – 36) in 2018. Estimated sex ratios in both models were variable, but were never significantly different from 0.5 (Appendix E; Appendix F). Estimated densities for each study area per year are shown in figure 7.

3.4 Detection

In Lierne, search effort had a positive effect on detection probability with increasing baseline detection probability in more searched areas (beta = 0.76; 95%CI 0.60-0.93; Fig. 5; Appendix E). In Skrim the detection probability was predicted by both search effort, road length and sex. Detection probability increased with both search effort (beta = 0.33; 95%CI 0.12-0.54) and road length (beta = 0.2; 95%CI -0.03-0.43), and detection probability was lower for males than for females (p0.male = -0.76; 95%CI -1.71-0.19; Fig. 6; Appendix F).

3.5 Space use

In Lierne, σ estimates were 1.55 km (95%CI 1.35 – 1.78) for females and 2.17 km (95%CI 1.91 – 2.46) for males (Fig. 5). Based on σ estimates using 95 % kernel density, mean home range size for females was 45 km^2 (95%CI 34 - 60) and 88 km^2 (95%CI 69 – 113) for males. In Skrim, σ estimates were 1,17 km (0,91 – 1,49 km) for females and 1,73 km (1,36-2,19 km) for males (Fig. 6). Estimated mean home range size for females was 26 km^2 (95%CI 16 - 42) and 56 km^2 (95%CI 35 - 91) for males.

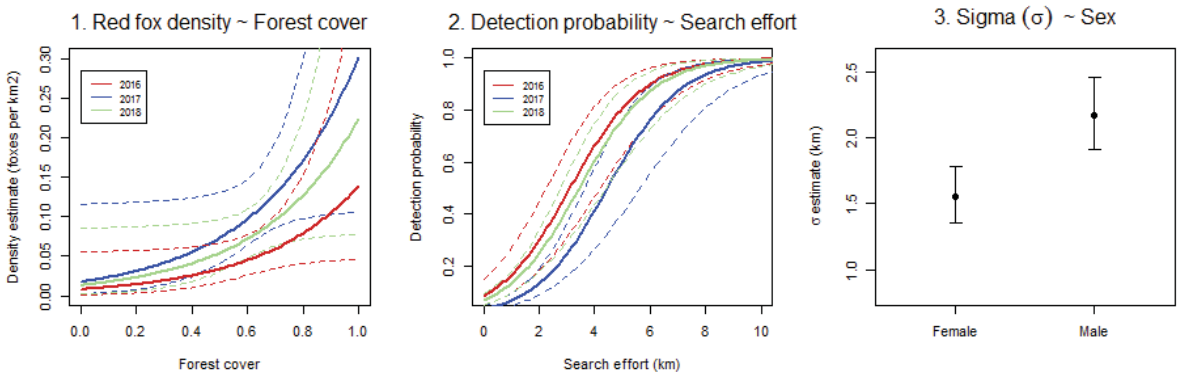

Figure 5: Plotted effects of covariates on the three main model parameters in the best oSCR-model by AIC for Lierne:
1. The effect of forest cover on density per year, shown with 95% confidence levels as dotted lines.
2. The effect of search effort on detection probability per year with 95% confidence levels as dotted lines.
3. The effect of sex on sigma (σ) with 95% confidence intervals.

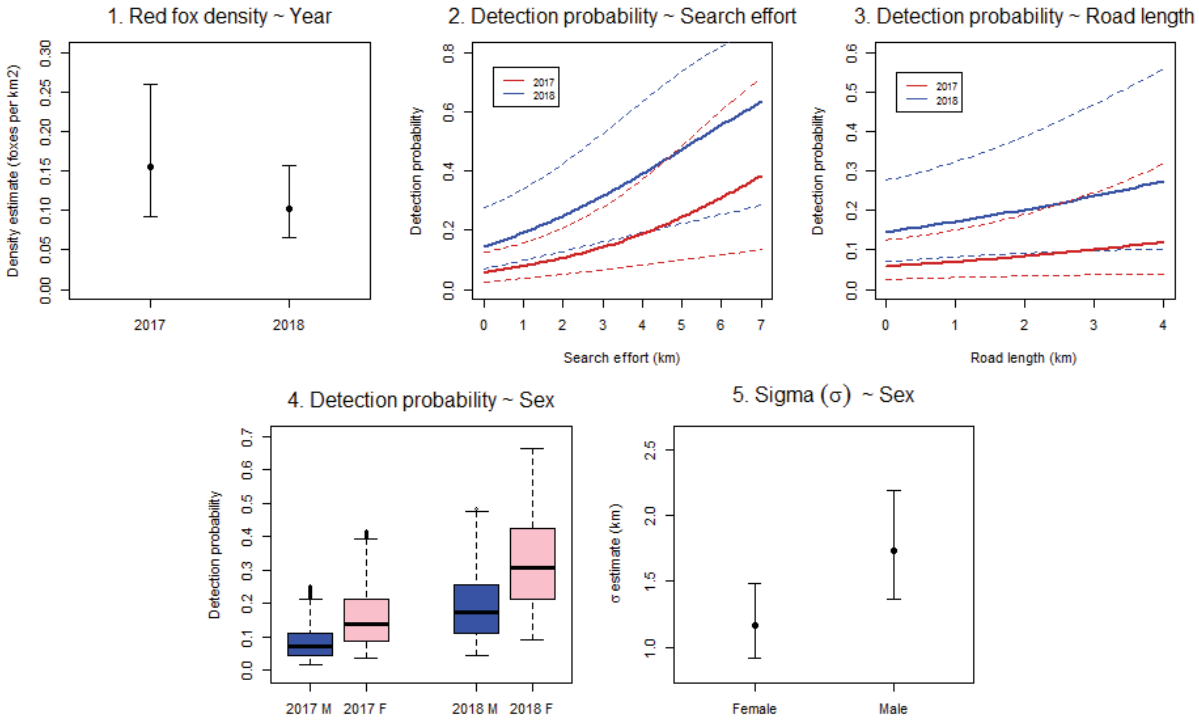

Figure 6: Plotted effects of covariates on the three main model parameters in the best oSCR-model by AIC for Skrim:
1. Density estimates per year with 95 % confidence intervals.
2. The effect of search effort on detection probability per year with 95% confidence levels as dotted lines.
   (The effect is shown for road length (scaled) = 0 and sex = male)
3. The effect of road length on detection probability by year with 95% confidence levels as dotted lines.
   (The effect is shown for search effort (scaled) = 0 and sex = male)
4. The effect of sex on detection probability per year (The effect is shown for road length = 0 and search effort = 0).
5. The effect of sex on sigma (σ) with 95% confidence intervals.

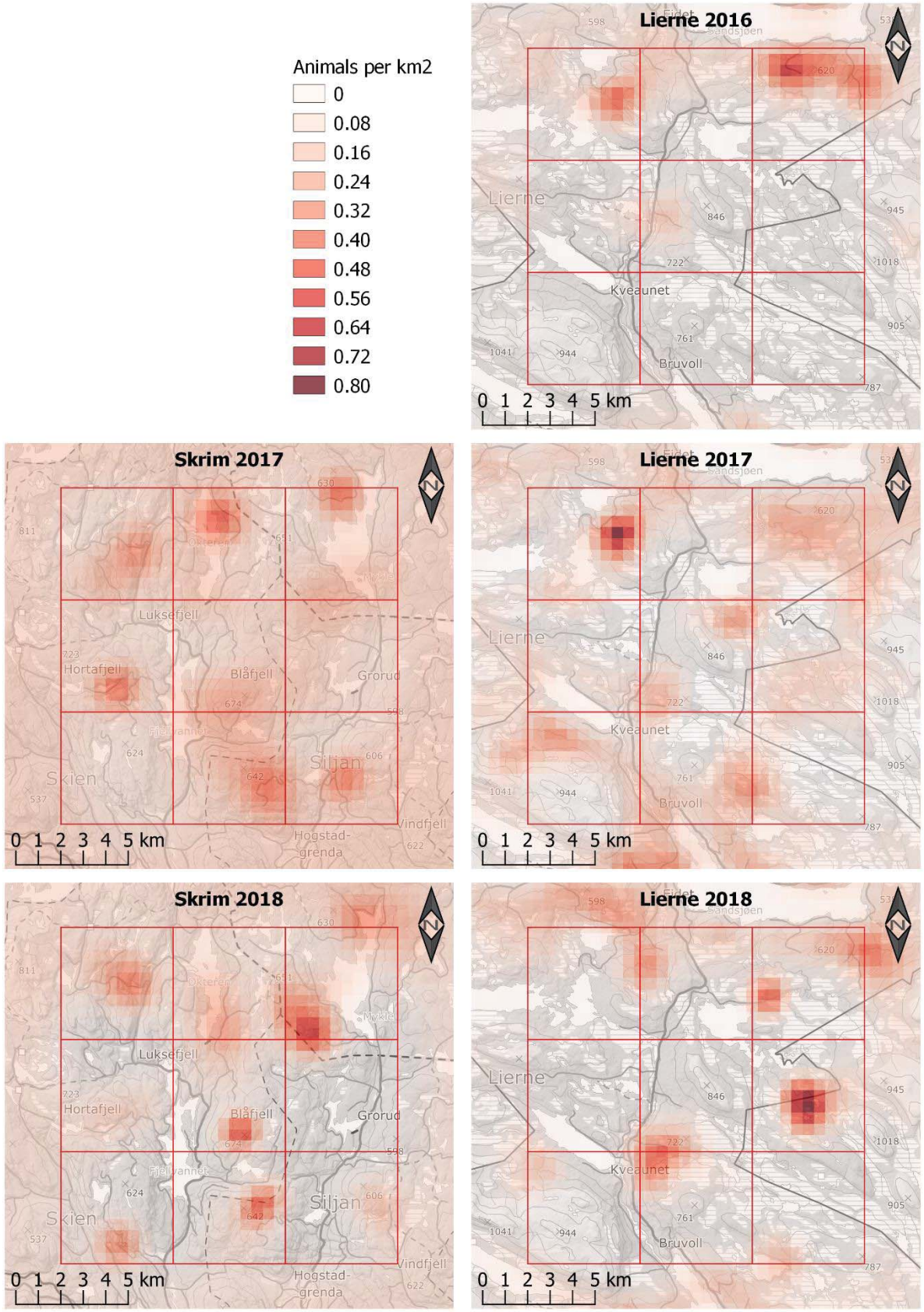

Figure 7: Estimated red fox density in Lierne in 2016, 2017 and 2018, and Skrim in 2017 and 2018, derived from respective oSCR-models.

4.0 Discussion

4.1 Data quality and reliability

From genetic analysis of non-invasively sampled red fox feces, urine and hair from two different study areas over a period of three years, individual foxes were successfully identified by genotype. From these density estimates could be generated from spatially explicit models that accounted for imperfect and variable detection. Considering the mean number of samples per individual, mean number of individuals detected and the spatial distribution of detectors in both study areas, the datasets contained sufficient spatial redetections for reliable estimates of the model parameters (cf. Royle et al. 2013). On average, 48 % of all samples collected contained DNA of sufficient quality for individual identification in accordance with a strict laboratory procedure. The proportion of successfully identified individuals was higher than previously reported success rates in a similar study of red fox in south-eastern Norway (36 %; Wegge et al. 2019). Considering that the samples collected were of varying type and quality, this is a decent success rate. Possible contributing factors could be strict procedure for handling and storing samples, and the use of established species-specific microsatellite markers in the genetic analyses.

4.2 Density and population size

The top model from each study area differed in terms of predictors for model parameters. Forest cover had a significant positive effect on red fox density in Lierne, but not in Skrim. The dataset from Skrim had a low sample size in both years and a lower mean number of samples per individual compared to Lierne. This may have resulted in a less robust model with insufficient evidence for the relationship between density and forest cover. However, these findings may also suggest that drivers of variation in red fox density may differ between areas. Differences in density may e.g. be driven by variation in vegetation and climate. The study area in Lierne is in the northern boreal vegetation zone with both forested and alpine areas and is in a climatic transitional zone between an oceanic and continental climate. In comparison, the study area in Skrim is in the southern boreal zone completely below the forest line and has an oceanic climate (Moen 1998). These differences make Lierne much colder compared to Skrim on average in the period in which sampling took place. Forests may therefore serve as important refuges for foxes in contrast to exposed alpine areas, and as such influence density.

Similarly, forests may also provide refuge for important prey species tied to boreal forest habitat, including voles, shrews and forest birds (Lundstadsveen 2010), and influence the abundance of food sources for the red fox. Additionally, in a concurrent study from Lierne, baited camera traps simulating carcasses were visited less often by foxes in forests than in alpine areas (Gomo et al. unpublished data). This may suggest a lower dependency on carcasses in forests compared to alpine areas, and indicate a high general resource availability for foxes in forests in Lierne. Also, because forest cover is less continuous in Lierne than in Skrim (Fig. 3; Fig. 4), much of the area consists of forest edge habitat where edge-effects may further facilitate concentration of red fox activity to forested areas (Kurki et al. 1998), and thus influence density.

As suggested by previous studies, other important drivers of red fox density are human land use and anthropogenic subsidies. Boreal forest landscapes with high human settlement density have been associated with higher abundances of red fox (Jahren et al. 2020). This is thought to be driven by increased availability of food sources of anthropogenic origin, thus increased scavenging opportunities (Jahren et al 2020; Rød-Eriksen et al. 2020). Due to a larger human population in adjacent areas in Skrim compared to Lierne, both human activity in general and the availability of anthropogenic food sources is presumably higher in Skrim. The higher density estimates for Skrim may therefore reflect an increased abundance of red fox due to anthropogenic food subsidies. This could possibly also have impacts on habitat use of foxes and thus explain why no effect of forest cover on density was found in Skrim.

Lastly, interannual variation in estimated red fox densities in both study areas may have been due to annual differences in recruitment and mortality rates. More specifically, e.g. variation in culling of foxes may have differed between the two study areas and years. However, some studies have reported that intensified culling has little effect on red fox abundances (Baker & Harris 2005; Kämmerle et al. 2019). The loss of culled individuals in the populations is suggested to largely be negated by rapid replacement of culled individuals through immigration of new individuals from adjacent populations (Heydon & Reynolds 2020; Kämmerle et al. 2019).

4.3 Detection

Search effort had a positive effect on detection in both areas. This was expected, as detection probability naturally should be higher in more searched areas than in less searched areas. Including search effort as a covariate on detection in the baseline model also ensured that search effort was adequately accounted for beyond the binary searched or not searched values in the model input. Session also influenced detection in both study areas, meaning detection probability differed between years. Because the detection of individual animals depended on the genetic analysis of NGS-samples, this may have reflected variation in genotyping success (Table 2) caused by year to year differences in weather and other environmental conditions that could have affected the quality of samples (see also section 4.5).

A difference between the study areas was the effect of road length on detection. Road length tended to positively affect detection in Skrim ($p = 0.086$; Appendix F), but not in Lierne. A possible explanation for this is that most of the sampling done in Skrim was along snow covered dirt roads, while most of the sampling done in Lierne was done along dedicated snow mobile and cross-country tracks instead of roads. Snow mobile tracks are by definition not included in the road length covariate, but may have a similar presumed effect on red fox space use and detection by providing cost-efficient traveling routes (Crête & Larivière 2003). This should, however, at least partially have been taken into account by the search effort covariate, that in essence make up the sampled snowmobile tracks.

4.4 Space use

Estimated mean home range sizes in each study area are comparable to recently reported mean home range using estimates from two GPS telemetry studies of red fox in similar habitat in Scandinavia (MCP estimates 61 km^2 and 52 km^2). However, the variation in reported home range estimates from both studies is significant (Svendsen 2016; Walton et al. 2017). Home ranges correlated with a productivity gradient in relation to both latitude and elevation. Home ranges were up to 4 times larger in less productive vegetation zones and at higher altitudes compared to more productive vegetation zones at lower elevations (Walton et al. 2017). The same picture can be seen here, where Skrim represents a more productive southern boreal forest at lower elevation with smaller home ranges (26 km^2 for females and 56 km^2 for males), compared to Lierne's less productive northern boreal forest, higher elevations and

larger home range estimates (45 km^2 for females and 88 km^2 for males). While neither Svendsen nor Walton et al. found significant intersexual differences in home range sizes, home range estimates reported here show that home range sizes of males were approximately twice the size of female home ranges in both study areas. Another study reported longer daily travel distances for males compared to females (Servín et al. 1991), of which could imply larger home ranges for males. Larger home range sizes for male foxes have also previously been reported in urban environments (White et al. 1996).

4.5 Non-invasive genetic sampling for SCR modeling

Analysis of DNA from urine and fecal samples has become a viable method for individual identification of animals (Hausknecht et al. 2007; Woodruff et al. 2015). Few hair samples were found in this study and had much lower genotyping success rates compared to urine and scats (Appendix A). This is consistent with previous studies that also reported paucity of samples found and the lack of follicles in found samples, from which DNA can be extracted (Vine et al. 2009). With fecal samples, many studies highlight the challenge of degradation of DNA obtained from the epithelial cells found in scats (Woodruff et al. 2015). Variation in degradation rates have been ascribed to differences in environmental conditions, sample age, diet and preservation methods (Panasci et al. 2011; Piggott 2005). The difference in proportion of successfully genotyped samples in Lierne is noticeably higher than in Skrim (Table 2). Although a large proportion of samples from Skrim contained sufficient DNA for species identification, less than half of the confirmed red fox samples contained sufficient DNA for genotyping (Appendix A; Appendix D). A possible explanation for this may be a difference in climatic conditions that impact the degradation of DNA. Evidence suggest that cold and dry conditions contribute to higher quality and quantity of fecal DNA (Woodruff et al. 2015). Due to its more continental climate, Lierne has a colder and more stable winter climate compared to Skrim, with temperatures predominantly at subzero throughout the sampling period (Moen 1998). Thus, the difference in genotyping success may have reflected differences in DNA degradation rates due to environmental differences.

One major advantage of using SCR modeling in conjunction with NGS is that repeated sampling, i.e. sampling over several occasions for sufficient redetections of individuals, is not required. This is because individuals can be identified from more than one sample at one or more detectors over the course of a single sampling session. This allows for sufficient spatial redetections, and thus successful estimation of model parameters (Kery et al. 2011). However, for studies of species with large home ranges where the study period is short, the scale parameter σ and area of estimated activity centers may be underestimated if the space use of identified individuals during the study period only represents a section of the animals' total home range (López-Bao et al. 2018; Walton et al. 2017). In this study the sampling period spanned more than a month for each study area and year, reducing the possibility of underestimation.

4.6 Considerations for future research

SCR and NGS methods can be resource intensive through both the high effort required in the field and costs of genetic analyses. Nevertheless, the combination of SCR and NGS methods provide a solid framework for not only estimating red fox density, but is also adequate for studying drivers of red fox density by identifying and testing spatially explicit covariates of interest. If applied in more areas and habitats, e.g. habitat mosaics of forest and farmland or arctic and alpine areas, SCR modeling may provide new insight into the relative importance of various drivers of density in red fox populations.

The implementation of a monitoring method for red fox based on NGS and SCR modeling would depend on appropriate sampling for sufficient spatial redetections of individuals for each survey. Although efforts may be done for more effective application of NGS methods, the SCR framework also has the potential for inclusion of other types of data in addition to NGS data. SCR modeling applied to large scale studies of large carnivores has in previous research also included DNA-samples from recoveries of dead animals for greater precision in density estimates (e.g. Bischof et al. 2019). Similarly, partially identified individuals can also be included for precision benefits (Augustine et al. 2018; Jimenez et al. 2019; Tourani et al. 2020). For red foxes, both data from locally shot individuals and camera trapping are commonly available. This data can be included in future applications of SCR modeling and provide precision benefits in density estimates.

Furthermore, SCR modeling can also be extended to open population analyses, with which one can study population dynamics over time, including mortality and recruitment rates, and inter-annual movements, i.e. immigration and emigration (Morin et al. 2016). If implemented at appropriate spatial scales, this also allows for the possibility to study landscape connectivity and resistance in relation to red fox dispersal and expansion (Sutherland et al. 2015). This information would give a solid basis for e.g. evaluating local management actions like culling initiatives, but may also provide information beneficial for the management of red fox on a bigger scale, e.g. in relation to its expansion into other habitats, like arctic and alpine areas.

5.0 Conclusions

The methods used in this study provide a reliable, but resource intensive approach for estimating density of red fox at appropriate scales for local management using non-invasive genetic sampling and spatial capture-recapture. Genetic analysis of NGS samples using species specific microsatellite markers allowed for successful genotyping and identification of individual foxes. This included sufficient redetections of individuals for SCR modeling. The SCR models not only estimated red fox density, but could also estimate the effects of both individual and spatially explicit covariates on the three main model parameters density, detection and space use. The estimates reported here are largely consistent with previous studies of red fox and support the application of NGS SCR methods for further research and development of monitoring methods. Further refinement of the models, where other types of data are included in addition to NGS, is expected to give more precise estimates.

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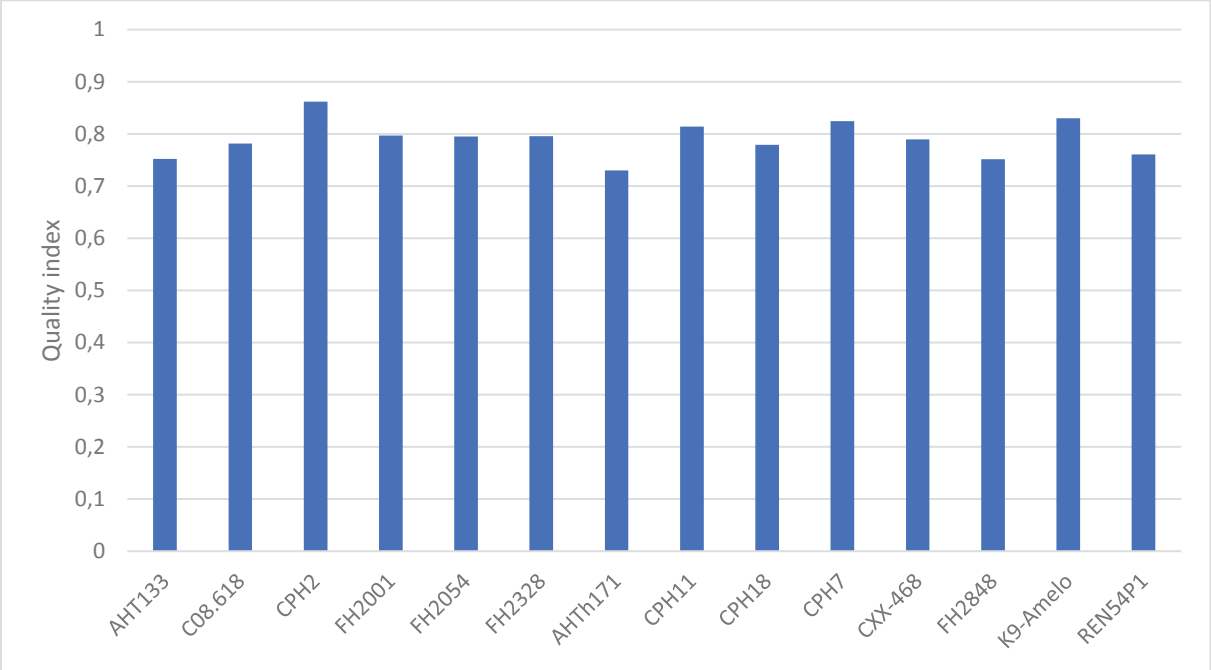
Appendices

Appendix A: Number of samples found per sample type compared to the number of confirmed red fox samples that were successfully genotyped for each study area and year. * = No urine preservatives were used in the pilot study.

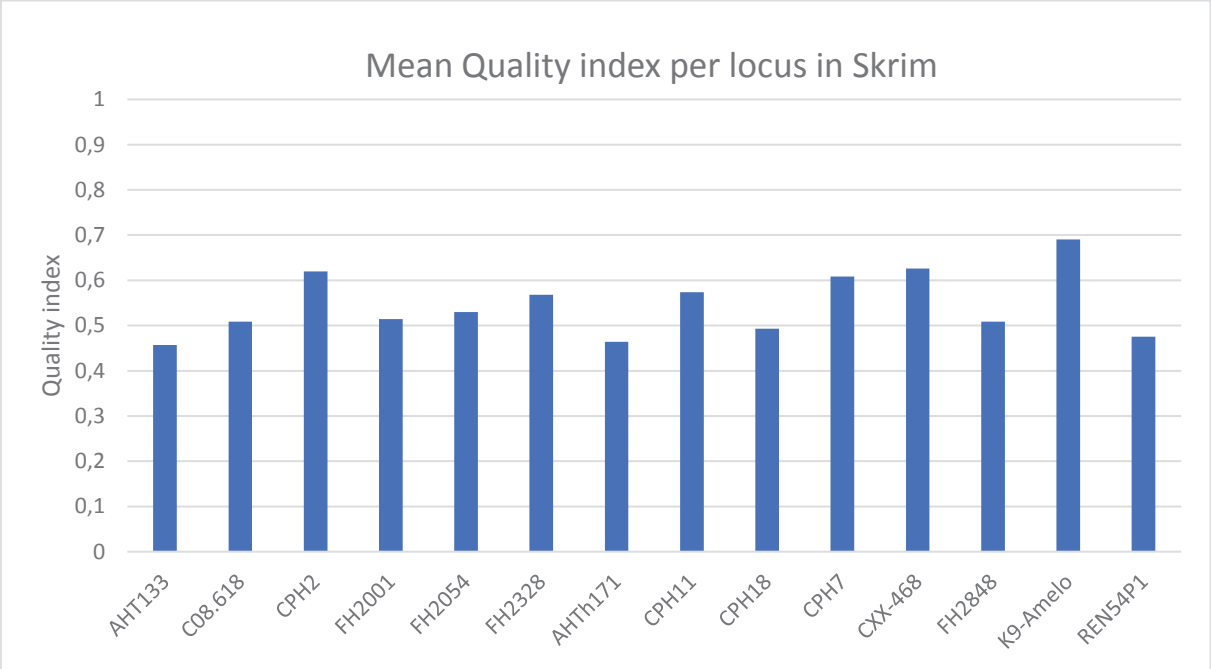
	Excrement		Urine		Hair	
	Found	Genotyped	Found	Genotyped	Found	Genotyped
Lierne 2016	60	56 (93 %)	100	*2 (2 %)	0	0 (N/A)
Lierne 2017	69	53 (77 %)	98	40 (41 %)	17	2 (12 %)
Lierne 2018	78	74 (95 %)	76	47 (62 %)	4	1 (25 %)
Skrim 2017	69	23 (33 %)	81	20 (25 %)	0	0 (N/A)
Skrim 2018	38	37 (97 %)	93	23 (25 %)	2	0 (0 %)
Total	314	243 (77 %)	448	130 (29 %)	23	3 (13 %)

Appendix B: Primer set concentrations in micromolar (µM) for the species specificity test and individual identification of confirmed red fox samples (MIX A + MIX B).

Species specificity		Multiplex MIX A		Multiplex MIX B	
Locus	**µM**	**Locus**	**µM**	**Locus**	**µM**
gulo	0.12	*AHT133*	0.16	*AHTh171*	0.08
H3R	0.36	*C08.618*	0.16	*CPH11*	0.12
pex	0.12	*CPH2*	0.16	*CPH18*	0.12
SRY	0.24	*FH2001*	0.64	*CPH7*	0.04
vul	0.12	*FH2054*	0.24	*CXX-468*	0.16
		FH2328	0.12	*FH2848*	0.16
		FH2457	0.80	*K9-AMELO*	0.24



Appendix C: Mean quality index (QI) per locus in Lierne.



Appendix D: Mean quality index (QI) per locus in Skrim.

Appendix E: Coefficient table for the top SCR-model in Lierne.

	Estimate	SE	z	P(>|z|)
p0.(Intercept)	-2.384	0.319	-7.483	0.000
p0.session2	-1.027	0.345	-2.973	0.003
p0.session3	-0.253	0.340	-0.745	0.456
t.beta.Ef	0.764	0.083	9.228	0.000
sig.(Intercept)	7.347	0.070	104.476	0.000
sig.sexmale	0.335	0.085	3.921	0.000
d0.(Intercept)	-6.183	0.973	-6.354	0.000
d.beta.Forest	2.827	1.431	1.975	0.048
d.beta.session2	0.772	0.329	2.343	0.019
d.beta.session3	0.473	0.332	1.427	0.153
psi1	-0.656	0.531	-1.235	0.217
psi2	-0.492	0.381	-1.291	0.197
psi3	0.077	0.402	0.191	0.849

Appendix F: Coefficient table for the top SCR-model in Skrim.

	Estimate	SE	z	P(>|z|)
p0.(Intercept)	-2.789	0.43	-6.486	0.000
p0.male	-0.758	0.484	-1.568	0.117
p0.session2	1.017	0.370	2.749	0.006
t.beta.Ef	0.331	0.106	3.114	0.002
t.beta.Rd	0.200	0.117	1.714	0.086
sig.(Intercept)	7.061	0.124	56.743	0.000
sig.sexmale	0.393	0.173	2.272	0.023
d0.(Intercept)	-3.249	0.264	-12.316	0.000
d.beta.session2	-0.424	-0.424	-1.250	0.211
psi1	0.054	0.464	0.116	0.908
psi2	-0.159	0.430	-0.371	0.711

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