Is non-invasive sampling of faecal pellets a reliable method to estimate rock ptarmigan (*Lagopus muta*) population density?

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Preface

This master thesis was carried out from 2012 to 2014 as a part of the Rock Ptarmigan Project coordinated by Telemark University College (TUC) in the department of Environmental and Health Studies. Fieldwork was executed between mid-March and mid-April 2013.

I am grateful to my supervisor Associate Professor Øyvind Steifetten for providing assistance and help in numerous ways, and I would also like to express my gratitude towards engineer Frode Bergan who supervised all laboratory work and provided abundant insight and assistance throughout the whole study. Thanks to engineer Karin Brekke Li and my friend Clara Moreno for their good help and points of view, and special thanks to my two lab- and fieldwork-mates Marte Darrud and Simen Storøy for enjoyable company and loads of laughter while tracking down faeces in the mountains and facing conundrums in the lab.

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Abstract

The rock ptarmigan (*Lagopus muta*) is relatively poorly studied worldwide, and little is known about its population status, which in Norway is based on hunting statistics. In order to develop proper and successful long-term management measures for any species, as well as to avoid poor management strategies, reliable data on population size and density over time is crucial. Importance should therefore be given to develop and choose the most reliable and effective estimation methods that provide results with firm precision. Faecal pellets were collected in spring from 75 snow roosts at the Lifjell plateau in Telemark County utilizing line transect surveys. All 75 samples were collected from a total of 35 observations, each containing from 1 to 5 snow roosts. Genetic analyses were used to investigate species of all samples, and 14 microsatellites were used in the individual identification. The species analyses resulted in 32 samples (43%) to originate from rock ptarmigan, and 43 samples (57%) to originate from willow grouse (*Lagopus lagopus*). The 32 rock ptarmigan samples originated from 28 different individuals due to re-sampling of one female which was sampled twice, and one male which was sampled four times. Estimated population density based on all 75 samples was calculated to be 2,66 birds per km^2, while estimates based solely on rock ptarmigan samples was calculated to be 0,99 rock ptarmigans per km^2. This suggest that estimating rock ptarmigan density by counting roosts will lead to a biased estimate, as the roosts are likely to originate from willow grouse. In Lifjell willow grouse habitat extend into rock ptarmigan habitat, leading to a mixing of roosts. Counting roosts therefore, presuming all are from rock ptarmigan, overestimates the rock ptarmigan population. Including genetic testing still provides a poor estimate, as it underestimates the population when compared to the counting of territorial males as performed in an unpublished study by Darrud & Storøy. This makes the method unattractive for field managers, as it provides erroneous estimates, is expensive, and demands laboratory knowledge. This, however, may not be the case in scenarios where no overlap between rock ptarmigan and willow grouse is present, either in other areas or at different times of the year.

1 Introduction

Genetic sampling is an important method in the study of wild animal populations, and lets researchers obtain DNA from a variety of sources, which can be used to identify for example the presence of species, count and identify individuals, determine sex and evaluate genetic diversity and population structures (Garshelis, 2006; Waits *et al*., 2005). Genetic sampling has for long been invasive, with disturbance and destruction of individuals being an inevitable part of the acquisition of genetic samples. This was due to the necessity of relatively large amounts of genetic material to extract sufficient amounts of DNA, amounts that non-invasive sampling could not provide (Taberlet *et al.,* 1999). Advances in biotechnological methods, however, have made it possible to duplicate DNA from minuscule quantities of genetic material (Sjøberg, 2013), which has lead to increased utilization of non-invasive sampling methods (Taberlet *et al.,* 1999).

Non-invasive sampling is sampling of biological material left behind by animals without the need for captivity or handling (Garshelis, 2006; Hájková *et al*., 2009; Luikart *et al*., 1999; Marucco, *et al*., 2011; Reiners *et al*., 2011; Solberg *et al*., 2006), and has had dramatically positive impacts on studies of threatened, endangered and elusive species (Borthakur *et al*., 2013; Rudnick *et al*., 2009). The method has for example been used in a study by Ebert (2011) to estimate population sizes of red deer and wild boar in Germany, by Janečka *et al*. (2008) to monitor populations of the shy snow leopard in Central Asia, by Green *et al*., (2007) to confirm family relations between mothers and calves of free-ranging Atlantic spotted dolphins, and by Faria *et al*. (2011) to confirm the presence of the rare mountain bongo in Kenya. The non-invasive genetic sampling methods hold great promise for wildlife biologists, but have been connected to low success rates, contamination concerns and high genotyping error rates (Waits *et al*., 2005).

The most common sources of DNA used in genetic testing are feathers, hair and faeces (Ebert, 2011). In winter rock ptarmigans (*Lagopus muta*) dig snow roosts for protection where they exploit the insulating properties of the snow (Kjelsaas, 2009; Pedersen & Karlsen, 2007). When abandoning the snow roost, a little mound of faeces can normally be found left behind by the bird (Figure 1) (Pedersen & Karlsen, 2007) for the sampler to easily collect. Sampling genetic material in winter has its advantages, as it most likely ensures little degradation of DNA if the conditions are cold and dry (Bergan, 2011). DNA is amplified using the polymerase chain reaction (PCR), and a unique genetic profile or fingerprint is obtained for each individual using microsatellite markers (Anderson *et al*., 2006; McKelvey & Schwartz, 2004; Mills *et al*., 2000), allowing for discrimination between individuals in a population (Ebert, 2011).

The rock ptarmigan is a high alpine species with a Holarctic distribution (Bech *et al*., 2009; Kvasnes *et al*., 2010; Martin *et al*., 2011), mostly found in the circumpolar region of the world (Fonstad *et al*., 2008; Quintela *et al*., 2010). It is the only herbivore bird species that spends its entire life cycle in alpine areas, and it is a popular game bird (Nilsen *et al*., 2012) Little is known about the population dynamics of rock ptarmigan in Norway (Borecha, 2011; Nilsen *et al*., 2012), and the species is overall poorly studied worldwide (Booms *et al*., 2011; Nilsen *et al*., 2012; Storch, 2007). The Norwegian Red List for Species lists the rock ptarmigan as "least concern" (Kålås *et al*., 2010) due to the species' widespread distribution (Nilsen *et al*., 2012), however, during the last decades, many grouse species and populations have experienced a decline in population sizes (Henden *et al*., 2011; Nilsen *et al*., 2012), and the potential distribution of rock ptarmigan is considerably larger than what hunting statistics suggests (Nilsen *et al*., 2012). In Norway the decline in population sizes has been noted by descending statistics in the hunt harvest (Nilsen *et al*., 2012).

Successful management and conservation of species depends on reliable estimates of population size and density (Bellemain *et al*., 2005; Garshelis, 2006; Green *et al*., 2007; Gwenaël *et al*., 2010; Marucco *et al*., 2011; Solberg *et al*., 2006), but there is no systematic monitoring of the rock ptarmigan populations

in Norway today. Present knowledge on rock ptarmigan population sizes and development is fragmented and poor, and a better understanding of the rock ptarmigan population status in Norway and the rest of Northern Europe is therefore necessary in order to engage and design the best conservation methods possible for the species in the coming future (Nilsen *et al*., 2012).

Estimates of rock ptarmigan population density are usually obtained from hunting statistics and line transect surveys (Bolstad, 2010), but in Norway estimates are based solely on hunting statistics (Borecha, 2011). Estimates of good quality are often difficult to obtain. The line transect survey method can provide accurate information about populations, but the quality of the line transect estimates can be sensitive to low sampling effort (Bolstad, 2010). Given that knowledge about a population's size, development and changes in time is necessary to ensure sustainable and long-term population conservation (Caizergues *et al*., 2003; Fuglei & Pedersen, 2008), importance should be given to develop and choose the simplest and most efficient population estimation methods that provide solid results and strong precision (Fuglei & Pedersen, 2008).

Figure 1: Rock ptarmigan snow roost with faeces and adjacent wing marks. (Photo: Rebecca Hornli Lundberg).

2 Material and methods

The main goal of this study has been to investigate the reliability of using non-invasive sampling of faecal pellets to estimate rock ptarmigan density in the Lifjell mountains, Telemark County, Norway. Lifjell is home to populations of both rock ptarmigan, willow grouse and black grouse, all which dig snow roosts and deposit analogous faecal pellets in the terrain. Both the willow grouse and the black grouse are subalpine species, but especially willow grouse is suspected to venture into alpine rock ptarmigan habitat in Lifjell and cause an overlap of habitats. It is therefore also of interest to investigate the amount of actual rock ptarmigan samples collected in the high alpine areas, as well as the amount of willow grouse and possible black grouse samples collected in rock ptarmigan habitat.

Samples have been collected from snow roosts in the field obtained by utilizing a line transect survey method. Collection of faeces was executed en route between points in the terrain, pre-determined by a grid, and divided by a distance of 500 meters. A hand-held GPS was used to navigate between points, and all observation-site coordinates were saved on a GPS.

2.1 Study Area

The samples used in this study were collected at the middle- and high-alpine plateau of Lifjell (Figure 2), a barren, isolated mountainous area located in Telemark County in Southern Norway (N59°30,000´ E8°58,810), approximately 2 hours by car from Oslo. Lifjell belongs to four municipalities; Bø, Notodden, Hjartdal and Seljord, and has an area measuring approximately 20.000 decare. The Lifjell plateau is dominated by alpine areas reaching above 1000 m.a.s.l, divided by subalpine lower areas and valleys. Lifjell has more than 20 summits reaching above 1100 m.a.s.l, the highest one at Mælefjell with a height of 1413 m.a.s.l. An isolated population of rock ptarmigan inhabits Lifjell, living in the alpine areas of the plateau. The closest areas with other rock ptarmigan populations are Blefjell in Buskerud County, approximately 40 kilometres away,

and Hardangervidda in Buskerud, Telemark and Hordaland Counties, approximately 80 kilometres away. Willow grouse also inhabits Lifjell, and both willow grouse and rock ptarmigan are hunted annually, often restricted by bag limits due to uncertainties about population sizes.

Figure 2. Location of the Lifjell Plateau in Telemark County, Southern Norway. (Source: Apple Maps).

2.2 Sampling

Faecal pellets from snow roosts were collected from an area of approximately 28 km^2 from mid-March to mid-April 2013. Cross-country skis were used in the search, and binoculars were used to look for snow roosts. Recently abandoned snow roosts were targeted, and only faeces assessed fresher than two weeks old was collected to avoid sampling DNA of poor quality as DNA degrade over time, especially when subjected to sunlight, heat, mould or humidity. Sample freshness was assessed visually and by inspecting time of last snowfall. Samples in old snow roosts have been subjected to several snowfalls, repeated thawing and freezing as well as prolonged periods of sun and wind. This generally worsens the aspect of the snow roost and faecal pellets, causing the pellets to dissolve and

rot. All samples were collected at 1000 m.a.s.l or above to increase the likeliness of collecting pellets from rock ptarmigan and not lower-dwelling gallinaceous species. Snow roosts were found separately or in clusters of up to 5 roosts, each roost containing from zero to approximately 40 pellets. All 75 samples were collected from a total of 35 observations. When found, the pellets were scooped into clean collection tubes using the tube's pertaining lids to avoid DNA-contamination from hands to faeces. Only pellets free of snow were collected to minimalize the water content in the pellets when thawed. Genetic material from each snow roost was kept separate at all times to avoid mixing of genetic material. Some roosts contained two types of faeces; dry, firm faeces in the shape of stools, and liquid faeces from the bird's appendix. Only stools were sampled, avoiding the faeces from the appendix which is more difficult to sample due to its liquid form when deposited in the terrain. Sample number, altitude, UTM coordinates and date was written on all collection tubes in the field, and this information was later applied to a table for an easy overview of sample data (Appendix 2). Without mixing the different samples, the collected stools were distributed on tissue paper and kept in a dry place to dry for at least two days. The samples were then stored in collection tubes in a dark and room-tempered place.

2.3 DNA extraction

DNA was extracted using the QIAamp® DNA Stool Mini Kit (50), Cat. No 51504, according to protocol "Isolation of DNA from Stool for Pathogen Detection" with minor modifications. One whole, dry faecal pellet was used from each sample, weighing from 136 - 446 mg, and each pellet was crushed to allow for maximum homogenization with the stool lysis buffer and thus maximum DNA extraction yield. The tubes with the extracted DNA were labeled, placed in numerical order in tube stands and stored in a fridge until further analysis.

2.4 Species identification

Species identity was investigated for all 75 samples to determine the number of samples originating from rock ptarmigan. This was necessary to find exact number of rock ptarmigan samples, as samples may originate not from only rock ptarmigan, but also other tetraonids such as willow grouse and black grouse (*Tetrao tetrix*). The species identification was done by incubating the extracted DNA from the 75 samples with species primers, and amplifying the DNA in a PCR thermocycler machine. The amplified DNA was applied to cast acrylamide gels along with a length size marker (ladder) and subjected to a gel electrophoresis. Gels were then stained with GelRed® dye to make visualization possible, and results were visualized in a UV-transiluminator and displayed on a computer screen in the program GeneSnap from SynGene. The results were printed for future reference. Species were distinguished by identifying bands of 212 base-pairs for rock ptarmigan and 154 base-pairs for willow grouse. This was done by comparing the visualized bands in the gel to the ladder. 32 samples originating from rock ptarmigans were selected for further analysis, which is to investigate how many samples, if any, originate from the same individual. Samples from other species than rock ptarmigan were discarded.

The PCR-mix consists of RNAse-free water, dNTP, magnesium chloride, buffer, bovine serum albumin (BSA), enzyme and species primers, which are mixed with DNA template from each of the samples with extracted DNA (Table 1). The DNA amplification process was performed in a PCR machine starting with a 10-minute denaturation at 94 °C and followed by 35 cycles of denaturation at 94 °C for 20 seconds, annealing at 56 °C for 30 seconds, and extension at 72 °C for 15 seconds. This is followed by a final extension at 72 °C for 7 minutes before storing the PCR product at 4 °C.

Table 1: PCR Reagents and concentrations for the species identification.

Reagents	Concentration in solution	µl per sample	Concentration in PCR reaction
RNAse free H_2O		12,05	
dNTP mix	2mM	2,5	0,2mM
Buffer	10x	2,5	1x
Lagsp3F	10pmol/ µl (µM)	1,0	0,4µM
Lag3R	10pmol/ µl (µM)	1,0	0,4µM
Mut3R	10pmol/ µl (µM)	1,0	0,4µM
$MgCl_2$	25mM	2,5	2,5mM
BSA	10 µg/µl	0,25	0,1 µg/µl
Amplitaq Gold LD	5U/µl	0,2	1U
DNA template		2,0	
Total PCR volume		25,0	
µl PCR mix for each sample		23,0	

2.5 Individual identification

To determine the number of individuals the 32 rock ptarmigan samples originate from, it is necessary to perform an individual identification test. This will determine whether samples are from 32 different birds, or if some individuals have been sampled more than once. As part of a pilot study conducted at Telemark University College, 24 microsatellites have been chosen among 42000 microsatellites isolated and sequenced at Cornell University in USA. 14 of these 24 microsatellites were chosen for their positive PCR-results to be used in the individual identification in this study. All microsatellites are based on genetic material from rock ptarmigan from the Hardangervidda National Park, Norway. A PCR-mix (Table 2) for 34 samples was made for each of the 14 selected microsatellites, enough for the 32 rock ptarmigan DNA samples, one positive control (extracted from liver) and one negative control to check for contamination. AmpliTaq Gold (low yield) was used with all 14 microsatellites. The microsatellites were incubated in a PCR thermocycler with the 32 rock

ptarmigan DNA-samples, the positive control and the negative control to amplify DNA. Before being run in the ABI (Genetic Analyzer) machine, PCR-product from all samples for all microsatellites were pipetted into the tubes of a PCR plate along with a mix consisting of 12 µl formamide and 0,5 µl ROX 500 size standard dye (ladder) (50-500 base-pairs). The PCR plate is heated to 94 °C in the PCR machine for 2 minutes and centrifuged before being placed in the ABI machine for analysis. Results were visualized on a computer screen in the program Genetic Analyser Data Collection Software. The number of base-pairs for each of the 32 DNA samples and the two controls was investigated with each of the 14 microsatellites. The base-pair signal numbers were applied to a table and alleles were defined for all microsatellites (Appendix 1). Alleles were compared between microsatellites and samples to discriminate between individuals as well as specify whether an individual has been sampled one or several times. One microsatellite provided signals to define only one allele, and was thus excluded from the study.

Table 2: PCR Reagents and concentrations for the microsatellite analysis.

Reagents	Concentration in solution	µl per sample	Concentration in PCR reaction
RNAse free H_2O		13,05	
dNTP mix	2mM	2,5	0,2mM
Buffer	10x	2,5	1x
$MgCl_2$	25mM	2,5	2,5mM
BSA	10 µg/µl	0,25	0,1 µg/µl
AmpliTaq Gold enzyme	5U/µl	0,2	1U
DNA template		2,0	
Total PCR volume		25,0	
µl PCR mix for each sample		23,0	

3 Results

67 kilometres were covered walking line transects, and a total of 220 kilometres were covered during the entire sampling period. This resulted in 35 observations of snow roosts, each observation containing from 1 to 5 roosts. A total of 75 samples were collected from these 35 observations. DNA was extracted and amplified for all 75 samples, and species and individual identification was successfully performed. 32 (43%) of 75 samples originated from rock ptarmigan (212 base-pairs). The remaining 43 samples (57%) originated from willow grouse (154 base-pairs). The individual identification resulted in 28 of 32 samples to be unique, assessed to originate from different individuals. 2 individuals were sampled more than once; one male was sampled 4 times, while 2 samples originated from the same female. The other 26 samples were from individuals only sampled once. These had at least 1 allele that they didn't share with any other individual in any of the microsatellites.

Population density was calculated for all 75 samples as well as only the 28 rock ptarmigan samples. The sample number (N = 75 or 28) was divided by the length walked (L = 67 kilometres), multiplied by 2 and then multiplied by the ESW (estimated strip width) value (210,02 meters). ESW was calculated using the program Density. The mean number of snow roosts in each observation was calculated dividing the total number of samples by the total number of observations.

Estimated population density for all 75 samples of rock ptarmigan and willow grouse was calculated to be 2,66 birds per km^2. Estimated population density for the 28 rock ptarmigan samples was calculated to be 0,99 birds per km^2. The mean number of roosts per observation was calculated to be 2,14.
The box plots show the median altitude at which rock ptarmigan and willow grouse dwell in spring (Figure 3). Rock ptarmigan have a median altitude of 1220 m.a.s.l., and willow grouse have a median altitude of 1112 m.a.s.l. A Mann-Whitney U test was run in Minitab to check for statistically significant difference

in altitude between rock ptarmigan and willow grouse (confidence interval at 95%). Altitudes between the species were significantly different (p = 0,0001).

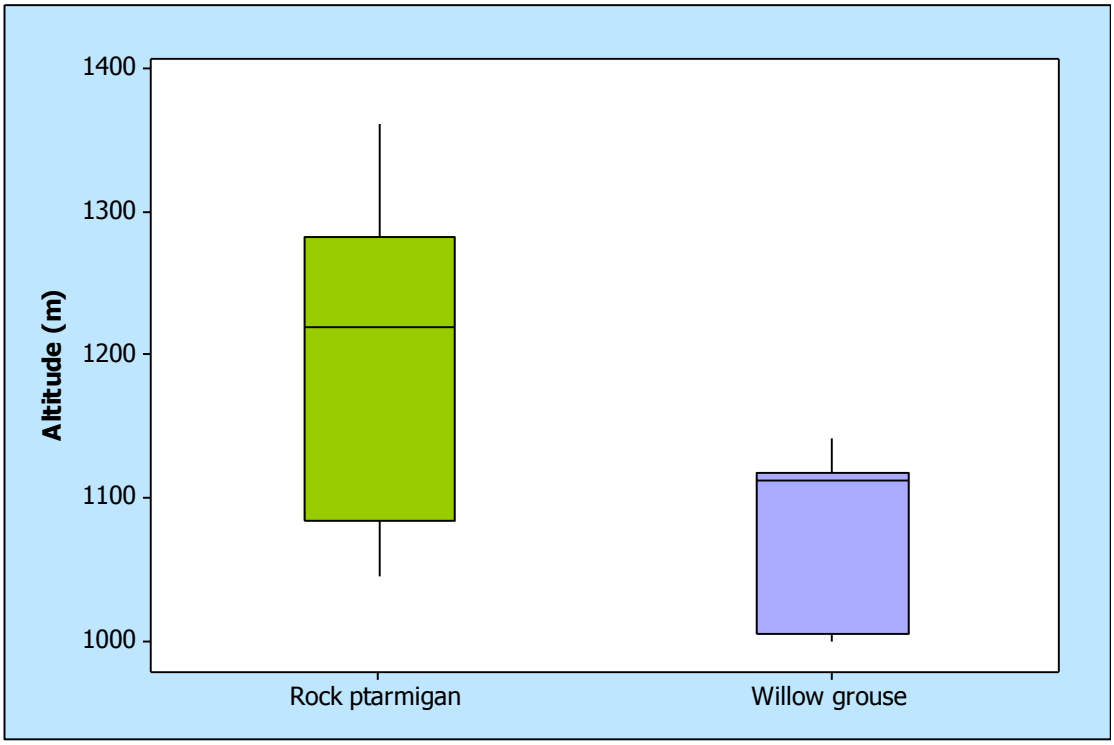

Figure 3. Box plots visualizing median altitudes for rock ptarmigan and willow grouse during spring in Lifjell, Telemark County.

4 Discussion

Despite collecting all samples in potential rock ptarmigan habitat, genetic testing revealed that the majority of the samples originated from willow grouse, while only the minority originated from rock ptarmigan. This suggests that the distribution of willow grouse in Lifjell, which is also more extensive than that of the rock ptarmigan, extends into rock ptarmigan territory, causing an overlap of habitats. This overlap leads to mixing of snow roosts and faecal pellets of the two species, and sampling of unwanted genetic material when only targeting one species is thus inevitable. Population estimation based on counting snow roosts in the field, presuming that all collected samples and observed snow roosts originate from rock ptarmigan, and furthermore that they all originate from different individuals of the species, heavily biases the field method, leading to a

serious overestimation of the rock ptarmigan stock. By including genetic testing, which enables for the discrimination between sample species, a different and lower rock ptarmigan population estimate was obtained, as all willow grouse samples were then excluded from the density estimate. However, including genetic testing still provides a faulty estimate, as it underestimates the population density when compared to the method of counting territorial rock ptarmigan males. In an unpublished study conducted by Darrud & Storøy from 2012 to 2014, a density of 0,78 rock ptarmigan cocks per km^2 was estimated by counting territorial rock ptarmigan males in Lifjell in Spring. Given that this estimate is based solely on males, it provides a higher density estimate than the genetic method used in this study, which provided an estimate of 0,99 rock ptarmigans of both sexes per km^2. The method used in this study thus fails twofold at providing a reliable density estimate of rock ptarmigan, as it overestimates the population by counting the total number of observed roosts, and underestimates it by including genetic methods. The applied method is therefore not attractive for field managers, as it not only provides erroneous population estimates, it also requires genetic testing to enable for the isolation of rock ptarmigan samples from other species. Furthermore, genetic testing does, in addition to being expensive and time consuming, require biotechnological knowledge as well as access to laboratory equipment. Field managers often do not possess this knowledge, nor do they have access to necessary laboratory facilities. Considering these factors and the unsatisfactory results of the density estimate, the applied method is not suitable to be adopted into the management of rock ptarmigan in Lifjell. This, however, may not be the case in areas where rock ptarmigan and willow grouse habitats do not overlap, or in other seasons when the ascent of willow grouse males in spring may be absent. In such a scenario, where the mixing of rock ptarmigan and willow grouse is absent, the applied method may prove both feasible and efficient. The applied method is thus a potentially efficient and good method, but it is not compatible with the ecology present at Lifjell in spring.

The choice of a minimum altitude at 1000 m.a.s.l for sampling rock ptarmigan proved sensible and promising, as no rock ptarmigan was sampled below

approximately 1050 m.a.s.l. However, this was complicated by the prominent presence of the willow grouse in the same area as rock ptarmigan samples were found. As visualized in the box plot, most willow grouse samples were collected on altitudes below the median value for the species, but still above 1000 m.a.s.l. which is potential rock ptarmigan territory. Running a Mann-Whitney U test revealed that there is a statistical ecological difference between the altitudes for rock ptarmigan and willow grouse in Lifjell, however, an overlap of habitats and mixing of species is still factual, causing the applied sampling method to fail.

A similar unpublished master thesis study conducted by Darrud & Storøy has shown that willow grouse males in Lifjell dwell with a median altitude approximately 100 meters higher up than females in spring. Suggested explanations for this is an attempt by the willow grouse males to increase the area in which their calls will be heard, as a way of marking territories and perhaps as an attempt to increase the likeliness of finding a female. Another suggested explanation for this ascent is habitat fragmentation and an upwards shifting of willow grouse habitat, possibly because of climate change. As the willow grouse inhabits subalpine areas, the upwards shifting of habitats may expand its territories, which may simultaneously lead to shrinkage of rock ptarmigan habitats. However, these suggestions are uncertain, but a better understanding can be achieved by increasing the knowledge and understanding of the rock ptarmigan and willow grouse population statuses in Lifjell.

Population estimates of rock ptarmigan in Lifjell from counting roosts and territorial males are low. As rock ptarmigan stocks have experienced a decline in population density in Norway the last decades, this drop in density may be more prominent and have a deeper impact on the isolated rock ptarmigan population on Lifjell. This is because isolated and small populations are more susceptible to negative pressure such as habitat fragmentation and competition with other species. Suggestions for future studies could be to investigate willow grouse and rock ptarmigan habitat use in Lifjell to achieve a better understanding of the species' choice of habitat, as well as investigate why and when the willow grouse, and especially willow grouse males, ascend to rock ptarmigan habitat during

spring in Lifjell. Other suggestions are the investigation of demographic changes and their effect on alpine species, as well as how the rock ptarmigan responds to the habitat overlap and upward shifting willow grouse population in Lifjell.

5 Conclusions

Sampling and analysing non-invasively collected faecal pellets is not compatible with the ecology present at Lifjell, and is not an ideal method for estimating rock ptarmigan in Lifjell, at least not in spring. Rock ptarmigan habitats overlap with willow grouse in Lifjell, thus increasing the likeliness of sampling willow grouse faeces. The method overestimates the population by counting roosts, and underestimates the population by including genetic testing compared to the method of counting territorial males in a study by Darrud & Storøy. In addition to provide erroneous population estimates, the method is also expensive and demands laboratory knowledge. However, this conclusion might not apply to scenarios where rock ptarmigan habitats are not inhabited by willow grouse. In such scenarios, the sampling of unwanted genetic material is less likely, and the applied method may prove feasible. It is unknown whether the overlap between rock ptarmigan and willow grouse is prominent only in spring or present around the year. This opens for possibilities for future studies.

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7 Appendices

Appendix 1: Base-pair signals for microsatellites and samples. B = Blank sample.

	Mut 1		Mut 2		Mut 3	
Sample no.	Signal (base-pairs)		Signal (base-pairs)		Signal (base-pairs)	
R 586	96		119		108	116
R 587	96		119		104	108
R 613	B		115	119	108	112
R 614	96		115		101	108
R 615	96				108	
R 616	96		111	115	108	112
R 617	96		115	119	109	
R 618	96		111	115	108	
R 619	96				108	116
R 620	96	104			100	
R 621	92		B		104	108
R 622	B		115	119	108	116
R 623	96		119		108	116
R 624	96		119		108	116
R 625	96	104	115	119	100	111
R 626	92		115		104	108
R 627	92	96	115	119	108	116
R 628	92		119	123	108	116
R 629	92	96			109	129
R 630	82	91	119		100	107
R 631	96		111	115	116	129
R 632	B				112	
R 638	96				108	
R 639	Error				108	
R 640	B		115	119	101	112
R 641	92		115	119	112	116
R 642	92				100	107
R 643	87				108	112
R 649	B		115		108	112
R 650	B				108	112
R 652	96				101	112
R 653	B				108	112
R 515	96		115	123	105	116
	Signal (base-pairs)	**Allele definition**	**Signal (base-pairs)**	**Allele definition**	**Signal (base-pairs)**	**Allele definition**
	82	A	111	A	100	A
	87	B	115	B	101	A
	91	C	119	C	104	B
	92	C	123	D	107	C
	96	D			108	C
	104	E			109	C
					111	D
					112	D
					116	E
					129	F

	Mut 4		Mut 6		Mut 9	
Sample no.	Signal (base-pairs)		Signal (base-pairs)		Signal (base-pairs)	
R 586	115	119	128		141	146
R 587	119	123	120		B	
R 613	115	119	120		137	141
R 614	115	119	120	128	141	145
R 615	115	123	128		124	133
R 616	119		120	128	141	
R 617	115	119	128		124	128
R 618	119		120	128	133	141
R 619	115	119	115	128	128	137
R 620	114	118	120	125	141	146
R 621	115	119	120	128	129	133
R 622	115	119	115	128	129	137
R 623	115	119	115	128	129	137
R 624	115	119	115	128	Empty PCRstrips	
R 625	115	122	115	119	141	146
R 626	115	119	120	128	129	133
R 627	119		120	128	137	150
R 628	119		119	128	125	137
R 629	119	122	127		260	
R 630	115	119	120	128	141	146
R 631	119	123	119	127	141	146
R 632	115	119	119		B	
R 638	114	118	126		137	146
R 639	115	119	B		135	139
R 640	119		119	123	B	
R 641	115		115	127	B	
R 642	114	118	B		141	146
R 643	115	119	318	322	137	146
R 649	114	118	119	128	125	137
R 650	119		119	128	B	
R 652	111	119	120	128	B	
R 653	115		120		B	
R 515	111	114	115	128	137	141
	Signal (base-pairs)	**Allele definition**	**Signal (base-pairs)**	**Allele definition**	**Signal (base-pairs)**	**Allele definition**
	111	A	115	A	124	A
	114	B	119	B	125	A
	115	B	120	B	128	B
	118	C	123	C	129	B
	119	C	125	D	133	C
	122	D	126	E	135	D
	123	D	127	E	137	E
			128	E	139	F
			318	F	141	G
			322	G	145	H

	Mut 12		Mut 13		Mut 16	
Sample no.	Signal (base-pairs)		Signal (base-pairs)		Signal (base-pairs)	
R 586	B		127		167	
R 587	131		B		163	
R 613	B		B		159	163
R 614	128	141	B		B	
R 615	B		144	153	163	167
R 616	127		157		B	
R 617	B		126	161	163	
R 618	126		B		159	
R 619	B		B		163	167
R 620	B		B		B	
R 621	131	142	B		167	171
R 622	126	141	127	157	163	167
R 623	B		B		163	167
R 624	126	141	B		B	
R 625	132		127		159	163
R 626	131	142	B		167	171
R 627	129	141	127	148	B	
R 628	128	144	157		159	
R 629	126		B		163	167
R 630	B		B		163	167
R 631	134	151	B		159	
R 632	129		B		159	163
R 638	B		B		159	167
R 639	B		B		159	
R 640	B		126		159	
R 641	B		B		163	171
R 642	B		148	153	163	167
R 643	120	126	B		159	167
R 649	B		144	157	163	167
R 650	B		B		163	167
R 652	B		B		159	163
R 653	129		B		163	
R 515	B		B		163	167
	Signal (base-pairs)	**Allele definition**	**Signal (base-pairs)**	**Allele definition**	**Signal (base-pairs)**	**Allele definition**
	120	A	126	A	159	A
	126	B	127	A	163	B
	127	B	144	B	167	C
	128	C	148	C	171	D
	129	C	153	D		
	131	D	157	E		
	132	D	161	F		
	134	E				
	141	F				
	142	F				
	144	G				
	151	H				

	Mut 18		Mut 21		Mut 22	
Sample no.	Signal (base-pairs)		Signal (base-pairs)		Signal (base-pairs)	
R 586	B		B		190	
R 587	B		178	194	190	
R 613	B		194		B	
R 614	B		186	194	B	
R 615	B		B		B	
R 616	B		203		B	
R 617	B		B		B	
R 618	B		187	212	B	
R 619	171	179	183	187	190	
R 620	179		178	198	B	
R 621	B		186	203	B	
R 622	B		182	186	191	
R 623	B		182	186	190	
R 624	B		182	186	B	
R 625	B		187	199	190	
R 626	B		186	202	B	
R 627	B		194	202	B	
R 628	179				190	
R 629	B		186		B	
R 630	179		B		B	
R 631	B		186	190	190	
R 632	171	179	150	197	190	
R 638	B		B		B	
R 639	B		183	191	B	
R 640	B		187		B	
R 641	B		B		190	
R 642	179		B		191	
R 643	B		199		B	
R 649	B		186		B	
R 650	B		186		B	
R 652	B		B		B	
R 653	B		191	212	B	
R 515						
R 516	179		183		B	
	Signal (base-pairs)	**Allele definition**	**Signal (base-pairs)**	**Allele definition**	**Signal (base-pairs)**	**Allele definition**
	171	A	150	A	190	A
	179	B	178	B	191	A
			182	C		
			183	C		
			186	D		
			187	D		
			190	E		
			191	E		
			192	E		
			194	F		
			197	F		
			198	G		
			199	G		
			202	H		
			203	H		
			212	I		

	Mut 23		Mut 24	
Sample no.	Signal (base-pairs)		Signal (base-pairs)	
R 586	182	193	B	
R 587	B		B	
R 613	B		B	
R 614	185	189	196	
R 615	B		196	
R 616	B		196	
R 617	B		B	
R 618	B		B	
R 619	B		196	
R 620	B		B	
R 621	B		B	
R 622	189	193	B	
R 623	B		196	
R 624	B		196	
R 625	185	193	196	
R 626	B		196	
R 627	B		196	200
R 628	B		196	
R 629	181		196	
R 630	B		196	200
R 631	B		196	
R 632	181	185	B	
R 638	B		B	
R 639	B		B	
R 640	B		195	
R 641	B		196	
R 642	181	193	196	200
R 643	193		B	
R 649	B		B	
R 650	No sizing data		B	
R 652	B		200	
R 653	189	193	196	
R 515				
R 516	B		B	
	Signal (base-pairs)	**Allele definition**	**Signal (base-pairs)**	**Allele definition**
	181	A	195	A
	182	A	196	A
	185	B	200	B
	189	C		
	193	D		

Appendix 2: Summary table with data on all the collected samples.

Sample no.	Analysis no.	Sampling date	Altitude (m.a.s.l.)	Species	Sex	Distance from line (m)
				L. muta (0) *L. Lagopus* (1)	Male (0) Female (1)	
FR 1	R 581	23.03.13	1005	1	0	76
FR 2	R 582	23.03.13	1005	1	1	76
FR 3	R 583	23.03.13	1005	1	1	76
FR 4	R 584	23.03.13	1005	1	1	76
FR 5	R 585	23.03.13	1005	1	1	76
FR 6	R 586	23.03.13	1045	0	0	130
FR 7	R 587	23.03.13	1045	0	1	130
FR 8	R 588	24.03.13	1059	1	0	57
FR 9	R 589	25.03.13	1000	1	0	95
FR 10	R 590	25.03.13	1000	1	0	95
FR 11	R 591	25.03.13	1000	1	1	95
FR 12	R 592	25.03.13	1000	1	0	95
FR 13	R 593	25.03.13	1004	1	1	125
FR 14	R 594	25.03.13	1004	1	0	125
FR 15	R 595	25.03.13	1004	1	1	125
FR 16	R 596	25.03.13	1004	1	1	125
FR 17	R 597	25.03.13	1046	1	1	57
FR 18	R 598	25.03.13	1022	1	1	83
FR 19	R 599	25.03.13	1017	1	1	60
FR 20	R 600	25.03.13	1010	1	1	40
FR 21	R 601	27.03.13	1112	1	0	123
FR 22	R 602	27.03.13	1113	1	0	111
FR 23	R 603	27.03.13	1112	1	1	170
FR 24	R 604	27.03.13	1112	1	1	242
FR 25	R 605	27.03.13	1112	1	0	242
FR 26	R 606	27.03.13	1112	1	0	242
FR 27	R 607	27.03.13	1112	1	0	242
FR 28	R 608	27.03.13	1118	1	0	176
FR 29	R 609	27.03.13	1118	1	1	176
FR 30	R 610	27.03.13	1118	1	0	176
FR 31	R 611	27.03.13	1118	1	0	176
FR 32	R 612	27.03.13	1118	1	1	176
FR 33	R 613	27.03.13	1112	0	0	196
FR 34	R 614	27.03.13	1228	0	1	68
FR 35	R 615	27.03.13	1328	0	0	112
FR 36	R 616	27.03.13	1220	0	0	58
FR 37	R 617	27.03.13	1220	0	0	58
FR 38	R 618	29.03.13	1362	0	0	226
FR 39	R 619	29.03.13	1286	0	0	160
FR 40	R 620	29.03.13	1286	0	1	160
FR 41	R 621	29.03.13	1286	0	1	160

Sample no.	Analysis no.	Sampling date	Altitude (m.a.s.l.)	Species	Sex	Distance from line (m)
				L. muta (0) *L. lagopus* (1)	Male (0) Female (1)	
FR 42	R 622	29.03.13	1291	0	0	217
FR 43	R 623	29.03.13	1282	0	0	239
FR 44	R 624	29.03.13	1282	0	0	239
FR 45	R 625	29.03.13	1282	0	1	239
FR 46	R 626	29.03.13	1282	0	1	233
FR 47	R 627	29.03.13	1283	0	1	101
FR 48	R 628	01.04.13	1084	0	1	81
FR 49	R 629	01.04.13	1084	0	1	81
FR 50	R 630	01.04.13	1084	0	1	81
FR 51	R 631	01.04.13	1084	0	0	81
FR 52	R 632	01.04.13	1084	0	1	81
FR 53	R 633	01.04.13	1130	1	0	24
FR 54	R 634	01.04.13	1130	1	1	24
FR 55	R 635	01.04.13	1130	1	1	24
FR 56	R 636	03.04.13	1112	1	0	137
FR 57	R 637	03.04.13	1112	1	0	137
FR 58	R 638	03.04.13	1221	0	0	152
FR 59	R 639	03.04.13	1221	0	0	152
FR 60	R 640	03.04.13	1314	0	0	72
FR 61	R 641	05.04.13	1142	0	1	75
FR 62	R 642	05.04.13	1142	0	1	75
FR 63	R 643	05.04.13	1142	0	0	75
FR 64	R 644	06.04.13	1142	1	0	37
FR 65	R 645	06.04.13	1142	1	0	37
FR 66	R 646	06.04.13	1142	1	0	37
FR 67	R 647	06.04.13	1142	1	0	37
FR 68	R 648	06.04.13	1142	1	0	37
FR 69	R 649	06.04.13	1219	0	0	6
FR 70	R 650	06.04.13	1219	0	0	6
FR 71	R 651	07.04.13	1097	1	0	113
FR 72	R 652	07.04.13	1050	0	0	52
FR 73	R 653	07.04.13	1050	0	0	52
FR 74	R 654	07.04.13	1088	1	1	109
FR 75	R 655	07.04.13	1088	1	1	109

Appendix 3: Allele definitions for microsatellites and samples.

Sample no.	Mut 1	Mut 2	Mut 3	Mut 4	Mut 6	Mut 9	Mut 12	Mut 13	Mut 16	Mut 18	Mut 21	Mut 22	Mut 23	Mut 24
R 586	D	C	C - E	B - C	E	G - I	Blank	A	C	Blank	Blank	A	A - D	Blank
R 587	D	C	B - C	C - D	B	Blank	D	Blank	B	Blank	B - F	A	Blank	Blank
R 613	Blank	B - C	C - D	B - C	B	E - G	Blank	Blank	A - B	Blank	F	Blank	Blank	Blank
R 614	D	B	A - C	B - C	B - E	G - H	C - F	Blank	Blank	Blank	D - F	Blank	B - C	A
R 615	D	Empty	C	B - D	E	A - C	Blank	B - D	B - C	Blank	Blank	Blank	Blank	A
R 616	D	A - B	C - D	C	B - E	G	B	E	Blank	Blank	H	Blank	Blank	A
R 617	D	B - C	C	B - C	E	A - B	Blank	A - F	B	Blank	Blank	Blank	Blank	Blank
R 618	D	A - B	C	C	B - E	C - G	B	Blank	A	Blank	D - I	Blank	Blank	Blank
R 619	D	Empty	C - E	B - C	A - E	B - E	Blank	Blank	B - C	A - B	C - D	A	blank	A
R 620	D - E	Empty	A	B - C	B - D	G - I	Blank	Blank	Blank	B	B - G	Blank	Blank	Blank
R 621	C	Blank	B - C	B - C	B - E	B - C	D - F	Blank	C - D	Blank	D - H	Blank	Blank	Blank
R 622	Blank	B - C	C - E	B - C	A - E	B - E	B - F	A - E	B - C	Blank	C - D	A	C - D	Blank
R 623	D	C	C - E	B - C	A - E	B - E	Blank	Blank	B - C	Blank	C - D	A	Blank	A
R 624	D	C	C - E	B - C	A - E	Empty PCR-strip	B - F	Blank	Blank	Blank	C - D	Blank	Blank	A
R 625	D - E	B - C	A - D	B - D	A - D	G - I	D	A	A - B	Blank	D - G	A	B - D	A
R 626	C	B	B - C	B - C	B - E	B - C	D - F	Blank	C - D	Blank	D - H	Blank	Blank	A
R 627	C - D	B - C	C - E	C	B - E	E - J	C - F	A - C	Blank	Blank	F - H	Blank	Blank	A - B
R 628	C	C - D	C - E	C	B - E	A - E	C - G	E	A	B	Empty	A	Blank	A
R 629	C - D	Empty	C - F	C - D	E	K	B	Blank	B - C	Blank	D	Blank	A	A
R 630	A - C	C	A - C	B - C	B - E	G - I	Blank	Blank	B - C	B	Blank	Blank	Blank	A - B
R 631	D	A - B	E - F	C - D	B - E	G - I	E - H	Blank	A	Blank	D - E	A	Blank	A
R 632	Blank	Empty	D	B - C	B	Blank	C	Blank	A - B	A - B	A - F	A	A - B	Blank
R 638	D	Empty	C	B - C	E	E - I	Blank	Blank	A - C	Blank	Blank	Blank	Blank	Blank
R 639	Error	Empty	C	B - C	Blank	D - F	Blank	Blank	A	Blank	C - E	Blank	Blank	Blank
R 640	Blank	B - C	A - D	C	B - C	Blank	Blank	A	A	Blank	D	Blank	Blank	A
R 641	C	B - C	D - E	B	A - E	Blank	Blank	Blank	B - D	Blank	Blank	A	Blank	A
R 642	C	Empty	A - C	B - C	Blank	G - I	blank	C - D	B - C	B	Blank	A	A - D	A - B
R 643	B	Empty	C - D	B - C	F - G	E - I	A - B	Blank	A - C	Blank	G	Blank	D	Blank
R 649	Blank	B	C - D	B - C	B - E	A - E	Blank	B - E	B - C	Blank	D	Blank	Blank	Blank
R 650	Blank	Empty	C - D	C	B - E	Blank	Blank	Blank	B - C	Blank	D	Blank	No sizing data	Blank
R 652	D	Empty	A - D	A - C	B - E	Blank	Blank	Blank	A - B	Blank	Blank	Blank	Blank	B
R 653	Blank	Empty	C - D	B	B	Blank	C	Blank	B	Blank	E - I	Blank	C	A
R 515	D	B - D	B - E (?)	A - B	A - E	E - G	Blank	Blank	B - C	Empty	Empty	Empty	Empty	Empty
R 516	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	B	C	Blank	Blank	Blank