***FINAL Report*:**

Developing a new approach to estimating the size of the UK capercaillie population using genetic material

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EXECUTIVE SUMMARY

This report constitutes the outcomes of the genetic work performed by the Royal Zoological Society of Scotland's WildGenes laboratory under contract number CCP_007, awarded by the Cairngorms National Park Authority. The RZSS WildGenes laboratory was contracted for a period of 4 months (Nov 2021-Feb 2022) to analyse 60 faecal samples collected by the Cairngorms Capercaillie Project (CCP). The samples were collected from lek sites during spring 2021 using 4 different collection and storage methods (Dried, ethanol, frozen, and swabs), 15 samples were collected per method. This pilot collection was conducted to inform the CCP on the most suitable method for future sample collection if conducting capture-recapture surveys using faecal samples at lek sites. The main findings are outlined below:

1. DNA extraction was attempted from all 60 samples, with those stored 'Dried' and 'Frozen' yielding the highest quantities of DNA.
2. All samples that could be genetically identified via the species ID test (n=43, 72%), were identified as capercaillie.
3. All samples that could be genetically sexed (n=33, 55%), were identified as male.
4. The samples collected and stored frozen (-20°C) outperformed the samples collected via the other methods across all tests reliant on intact capercaillie DNA, 87% were identified as capercaillie, 100% were identified as male, 47% could be genotyped at ≥3 microsatellites.
5. We therefore advise that samples are collected and stored using the 'frozen' protocol in future surveys targeting capercaillie DNA from faecal samples.
6. Even the most successful method only contained one sample that produced genetic data on all tests. This is not surprising given that faecal samples contain degraded DNA and inhibitors, but we advise that any future survey uses a genotyping method designed for degraded DNA or sampling effort is increased with the expectation that only a subset (<40%) of collected samples will prove useful.

INTRODUCTION

Populations of Western capercaillie, *Tetrao urogallus,* in Scotland, have been declining since the mid-1970s (Watson and Moss 2008). The most recent national survey, conducted by the Royal Society for the Protection of Birds (RSPB) and Scottish Natural Heritage (SNH) during the winter of 2015-16, estimated that 1114 (95% Confidence Limits: 805-1505) individuals remained in the country (Wilkinson *et al.* 2018). This is extremely concerning as it was estimated that 20,000 capercaillie were present in Scotland in the 1970s, therefore a decline of more than 90% of the population has occurred in the last 50 years. This is not the first time that capercaillie numbers have declined, having been reintroduced in 1837 to the UK after extirpation during the 18th century. More recent anecdotal evidence has suggested the current declines have been continuing unabated since the last national survey, elevating concerns that capercaillie will become locally extinct for a 2nd time in the UK.

Multiple factors have been highlighted as possible reasons for capercaillie declines, including collisions with deer fences (Moss *et al*. 2006), wetter summers (Coppes *et al*. 2021), predation (Baines *et al*. 2004) and human disturbance (Coppes *et al*. 2017). If successful, genetic lek surveys stand to help alleviate the latter, by reducing the residual disturbance caused by distance sampling and observation methods. Both methods follow strict protocols to minimise disturbance, but scope exists to further reduce this if genetic methods are adopted. Genetic material can be collected later in the day once birds have dispersed. In contrast, observation methods require surveyors to be present when birds are also present and therefore more vulnerable to disturbance. The current method used to estimate population size during the Scottish national survey is also labour intensive; it requires an important level of organisation and many volunteers working over several months, while the results produced have a limited precision and large confidence limits. This can be problematic especially when the goal is to detect significant changes in population size over time at a local scale. While it is currently vital to keep monitoring the population using field surveys, other techniques could bring new insights. Genetic tools have been used on a range of species to produce more robust population size estimates and can answer other questions of management concern. Genetic tools are already aiding management of capercaillie in other European populations (Alda *et al*. 2013; Jacob *et al*. 2010; Rösner *et al*. 2014; Segelbacher and Storch 2002). Therefore, establishing robust new techniques are likely to decrease the uncertainty currently associated with the population estimates and reduce the risk of disturbance.

This report outlines the initial feasibility of using a genetic approach for the population monitoring and management of capercaillie on lek sites in the Cairngorms National Park by assessing faecal samples collected by the Cairngorms Capercaillie Project team. Collection of faecal samples are a less invasive method of collecting DNA from a focal species and can be extremely useful for elusive species or those that are more prone to disturbance. However, the DNA quality and quantity obtained from faecal samples can be highly variable between species (Taberlet & Luikart 1999) and it is advisable that trial studies are conducted before decisions on their use are made (Taberlet *et al.* 1999). The Royal Zoological Society of Scotland's WildGenes laboratory has therefore been contracted to conduct the following:

1) Attempt to extract DNA from 60 faecal samples collected via 4 different methods.
2) Screen the DNA obtained by running genetic tests reliant on intact capercaillie DNA.
3) Identify the species associated with each faecal sample using mitochondrial DNA.
4) Test nuclear DNA quality by performing a sexing test and microsatellite amplification
5) Produce a report with recommendations on the optimal storage method that can be used for the pilot lek study in 2022.

This report is a summary of the analyses conducted and provides advice for the collection, storage and potential use of faecal samples during future collections.

METHODS

The RZSS WildGenes lab received a delivery of samples on 21/09/21 that had been collected from 60 faecal samples via four collection/storage methods (Table 1).

Table 1. *Summary of the samples received by the RZSS WildGenes lab.*

Item	Count
Swab in Buccal fix, DNA preservation buffer (2ml tube)	15
Dried faecal sample with silica (50ml tube)	15
Frozen faecal sample (50ml tube)	15
Frozen faecal sample in ethanol (50ml tube)	15

Each sample was labelled with a unique identification number (CAPXXXX) and placed in a -20°C freezer for long term storage (Figure 1). The collection/storage methods are referred to as 1) **Swab** - the faecal sample was swabbed in the field and the swab stored in Buccal fix, a DNA preservation buffer 2) **Dried** – a whole faecal sample was placed in a 50ml tube containing silica beads 3) **Frozen** – a whole faecal sample was placed in a 50ml tube in the field and then stored in a -20°C freezer as soon as possible, and 4) **Ethanol** – a whole faecal sample was placed in a 50ml tube, ethanol was added in the field and then stored at -20°C as soon as possible. Each of the collection methods required slightly different DNA extraction procedures as described below.

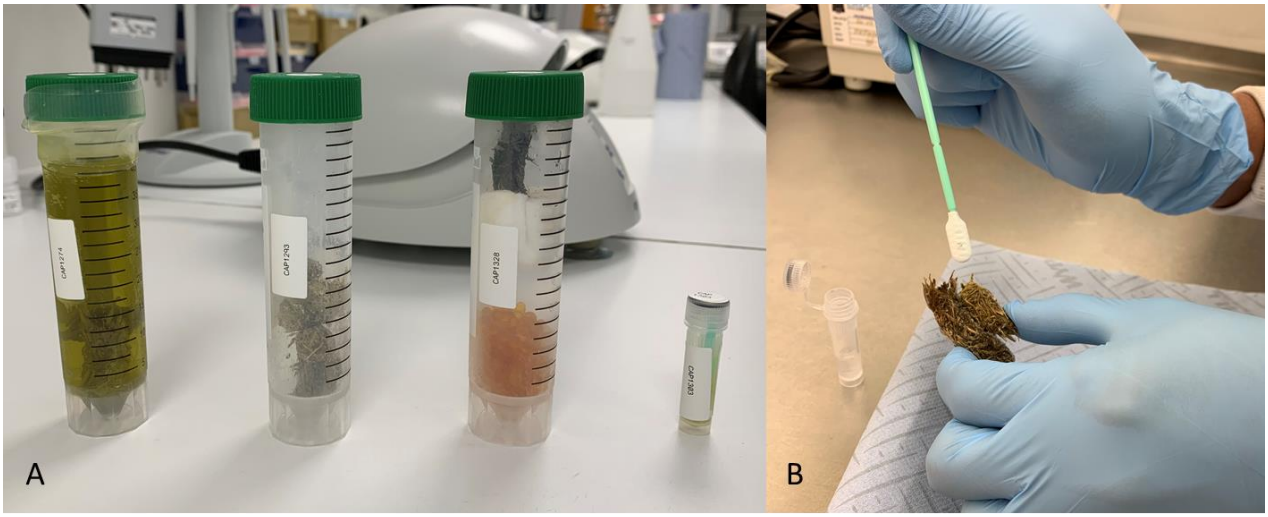

Figure 1. *The 4 sample types (L-R - Ethanol, Frozen, Dried and Field Swab) received by the RZSS WildGenes lab (A) and an example of the swabbing that was conducted in the lab pre-extraction for the dried and frozen samples (B).*

A. DNA extraction and quality control

DNA extraction was attempted for all 60 samples. A QIAamp® Fast DNA Stool Mini Kit was used for every extraction following the manufacturers protocol, however different preceding steps occurred for each sample type as follows:

1) **Swab** – the swab was turned around (swab head end up) and the tube centrifuged for 1 min at 4000rpm. The supernatant was pipette in to a clean 1.5ml flip-top tube. Then half an InhibitEx tablet was added to the tube and vortexed for at least one minute. The sample was left for 1 minute at room temperature to allow the inhibitors to be absorbed and then centrifuged for 3 minutes at full speed (14.5x1000 rpm) to pellet the stool and tablet particles. Then pipette

supernatant in to new 1.5ml flip-top tube. The supernatant was then used for the extraction which began with the proteinase K digestion.

2) **Dried** – the external surface of the faecal sample was swabbed with a cotton swab dipped in buccal fix. The head of the swab was snapped off and placed in a 2ml tube containing 500µl of buccal fix. The same steps as for the swab method above were then followed.
3) **Frozen** – the sample was partially defrosted and the external surface of the faecal sample was swabbed with a cotton swab dipped in buccal fix. The head of the swab was snapped off and placed in a 2ml tube containing 500µl of buccal fix. The same steps as for the swab method above were then followed.
4) **Ethanol** – the sample was vigorously shaken to dissipate the faecal sample. Then 3ml of the mix was taken in stages using a 1000µl filter tip (cut end off pipette tip to make pipetting easier). Centrifuge at full speed (14.5x1000 rpm) for 5 minutes and pipette off and discard the ethanol. Centrifuge again at full speed for 1 minute and pipette off any remaining ethanol. Place the tube containing the faecal matter on to a thermoblock at around 35°C with the lid open until the sample is dry. Add 1ml InhibitEx buffer – vortex for at least 1 min (until the sample has homogenised with the buffer – this can take some time if the sample is very dry). Leave to stand for 2 mins and then centrifuge for 1 min at full speed (14.5x1000rpm). Then pipette supernatant in to new 1.5ml flip-top tube. The supernatant was then used for the extraction which began with the proteinase K digestion.

The amount of extracted DNA obtained for each sample was then quantified using a Nanodrop® ND-1000 Spectrophotometer (ThermoScientific). This allowed us to test for the presence or absence of DNA in our extraction, however, with faecal samples this method is not a useful indicator of the amount of capercaillie DNA within the sample, as DNA could also have been extracted from, gut-bacteria, fungus or the dietary species. We therefore performed subsequent quality checking of the DNA using the three following methods, 1) species ID via mitochondrial DNA, 2) an avian sexing test and 3) microsatellite genotyping using capercaillie specific primers. The success of each of these subsequent methods is dependent on the presence of intact DNA from capercaillie.

B. Species identification

To identify whether the faecal samples originated from capercaillie, all samples were sequenced at a small region of the mitochondrial DNA (mtDNA) for species identification. A polymerase chain reaction (PCR) was used to amplify a region of the cytochrome B (CytB) mitochondrial gene using the previously published MCB primers (MCB_398_F and MCB_869_R - Verma and Singh 2002). The CytB gene is used extensively as a species identification tool, with high power to discriminate between species and sometimes populations. The amplified fragments (~450 bp) were sequenced on an ABI3730XL capillary sequencer then trimmed and aligned within the software Geneious Prime® 2019.0.4. Sequences with a high-quality base pair percentage (HQ) below 80% were considered unreliable and discarded from further analysis. The Geneious Prime® software was also used to run a Basic Local Alignment Search Tool (BLAST) analysis using the NCBI database, in order to search for similar sequences online. This last step allows rapid species identification.

C. Sex identification

Sex identification was achieved using two avian genetic tests, one published by Jensen *et al.* (2003) and the other by Lee *et al.* (2010). Both use the CHD-1 gene that is located on both avian sex chromosomes. Birds have two sex chromosomes, Z and W, and opposite to mammals, male birds are homogametic, meaning they have two copies of the Z chromosome, and females are heterogametic, having a single copy of a Z and a W chromosome. The CHD-1 gene is different between the Z and W chromosomes allowing us to use it to identify the sex of each sample. PCR amplification was done using primers tested by Jensen *et al.* (2003) and Lee *et al*. (2010). The results of the PCR were visualised via agarose gel electrophoresis. A male sample was identified by the presence of a single band (Z) on the gel, while a female sample was identified by the presence of two bands (ZW) (Solari 1993) (Figure 2). A known male and known female positive control, and a blank negative control were included during each PCR.

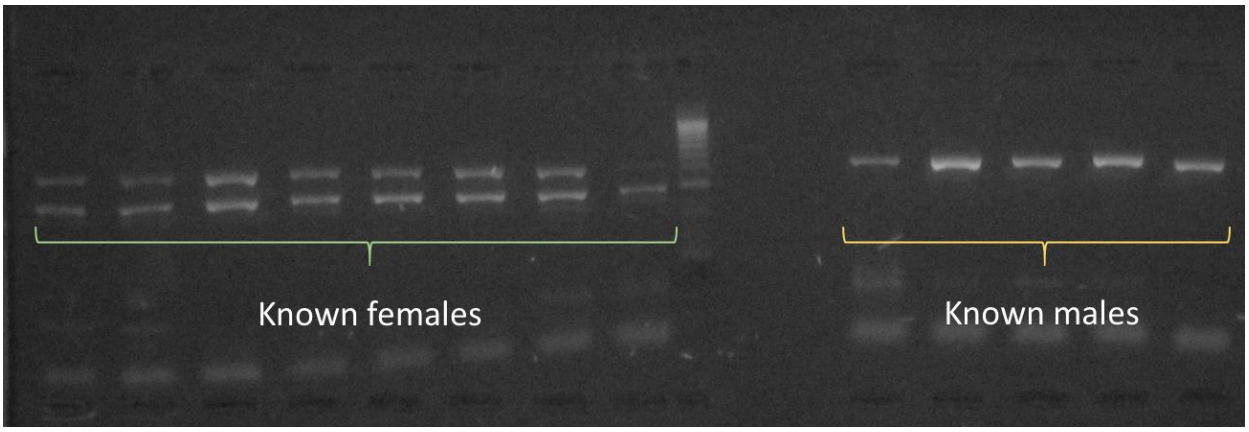

Figure 2. *Gel image of a sexing test using the CHD-1 gene. The males are identified by the presence of a single band and the female by the presence of two bands. The ladder (in centre) is used to determine the molecular weight of each band.*

D. Microsatellite genotyping

A DNA quality check via gel electrophoresis provides limited information when working with DNA extracted from faecal samples as the majority of DNA is likely from the gut microbiota or from diet species. To evaluate the integrity of the capercallie nuclear DNA that has been extracted we have used a panel of microsatellites that are specific to capercaillie and will therefore only amplify capercaillie DNA. This will provide a proxy for the success of any downstream individual identification methods that rely on intact nuclear DNA. The WildGenes lab has previously used two panels of microsatellites to genotype capercaillie samples. Here, we use one of these panels to evaluate the quality of DNA extracted from the 60 faecal samples collected via 4 different methods. The panel uses primers labelled with fluorescent dyes and amplifies 5 microsatellite loci in multiplex that have previously been identified for use in capercaillie research (Segelbacher *et al*. 2000). It is the panel B loci as used in Fletcher *et al.* (2018); TUD1, TUD3, TUT3, TUD4 and TUD7.

DNA extracts were PCR amplified and analysed on an ABI 3730 Sequencer (Applied Biosystems). All samples were run in triplicate and genotyped using Geneious Prime®. The loci details including the expected allele size range can be found in Table 2.

Table 2. Summary of the microsatellite loci to be used to evaluate nuclear DNA extraction success.

Locus name	Flourescent dye	Primer sequence	Allele size range
TUD1	6FAM	F:ATTTGCCAGGAAACTTGCTC	165-173
		R:TGAACATAACCTTTGCCTCC	
TUD3	PET	F:TCCAAGGGGAAAATATGTGTG	120-122
		R:TGGCTCTATTAAGGCTGCTG	
TUT3	6FAM	F:CAGGAGGCCTCAACTAATCACC	103-111
		R:TGAGGGATTTATGCATGCTGC	
TUD4	VIC	F:TCCCAAGGAGAGACAAAAGG	93-125
		R:GGGAGGACTGTGTAGGAGAGC	
TUD7	VIC	F:TGACACTGGGGTCATTAGGC	157-161
		R:CCTCTGGTGAAAGGGAATGG	

RESULTS

A. DNA extraction

DNA was successfully extracted from all 60 samples. The amount of DNA according to the Nanodrop measurements were significantly different between the collection methods (Figure 3, One-way ANOVA: df=3, F=19.91 , p<0.001). All collection methods were significantly different except for the ethanol and swab method which were both equally low, tested using a Tukey multiple comparison test. The highest quantity of DNA was obtained from the 'dried' faecal samples with the 'Frozen' samples also producing high yields.

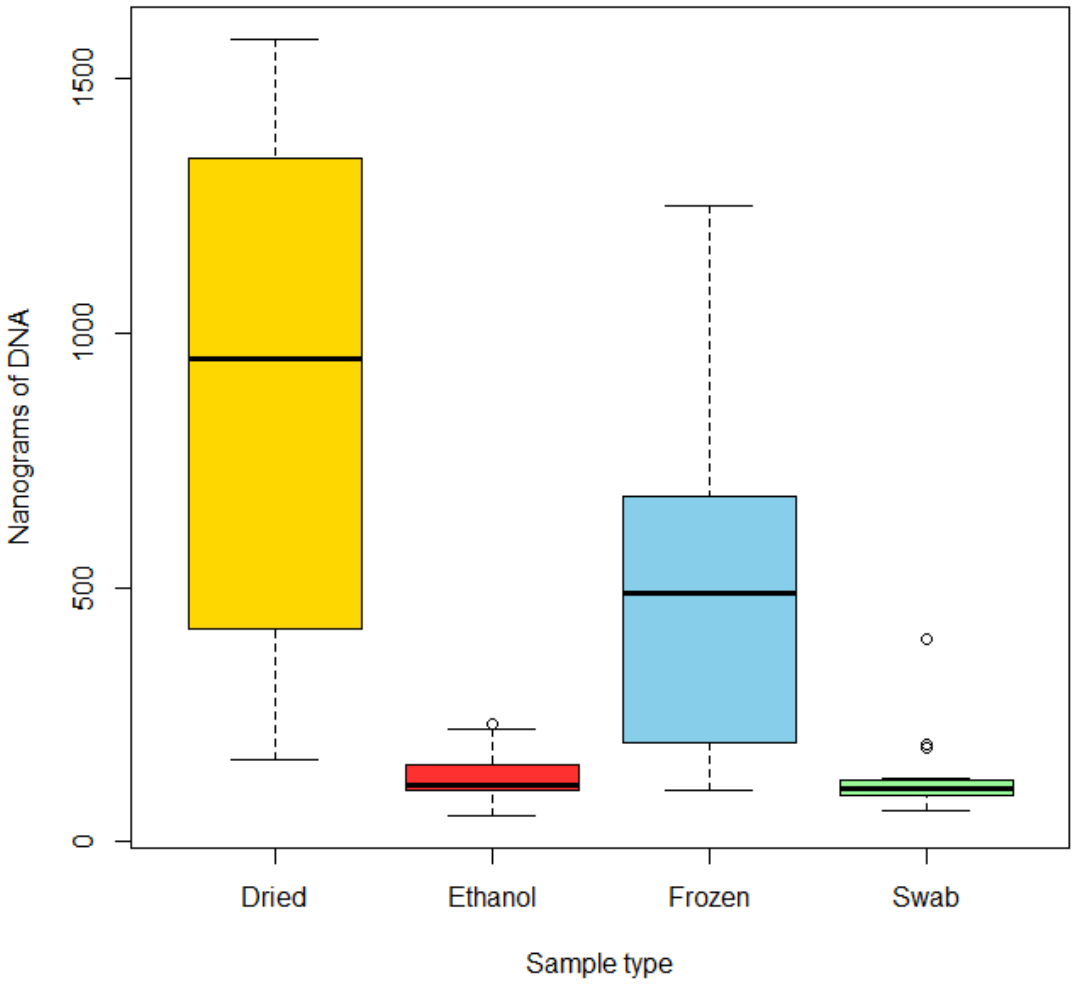

Figure 3. *A comparison of the amount of DNA extracted (in nanograms) from samples collected via the four different methods. There were 15 samples collected via each method.*

B. Species identification

Species identification was attempted on all 60 samples using cytochrome-b primers that will amplify DNA from vertebrate species (including mammals and birds). Eleven (18%) of the DNA extracts failed to amplify suggesting there is no (or too little) vertebrate DNA within the samples, or possibly a high number of PCR inhibitors. There were significant differences between the collection methods that affected the ability to identify a sample (Chi-squared test, $\chi^2 = 20.809$, df = 6, p-value = 0.002). All samples that failed to amplify were collected either using the Ethanol (n=8) or Swab (n=3) method, which were the sample types that contained the lowest amounts of DNA following extraction (see previous section). Amplified DNA from the other 49 samples were processed for sanger sequencing. The majority (43/49, 88%) were sequenced successfully and of these, all were an exact match to capercaillie. All results are summarised in Figure 4 and Appendix 1.

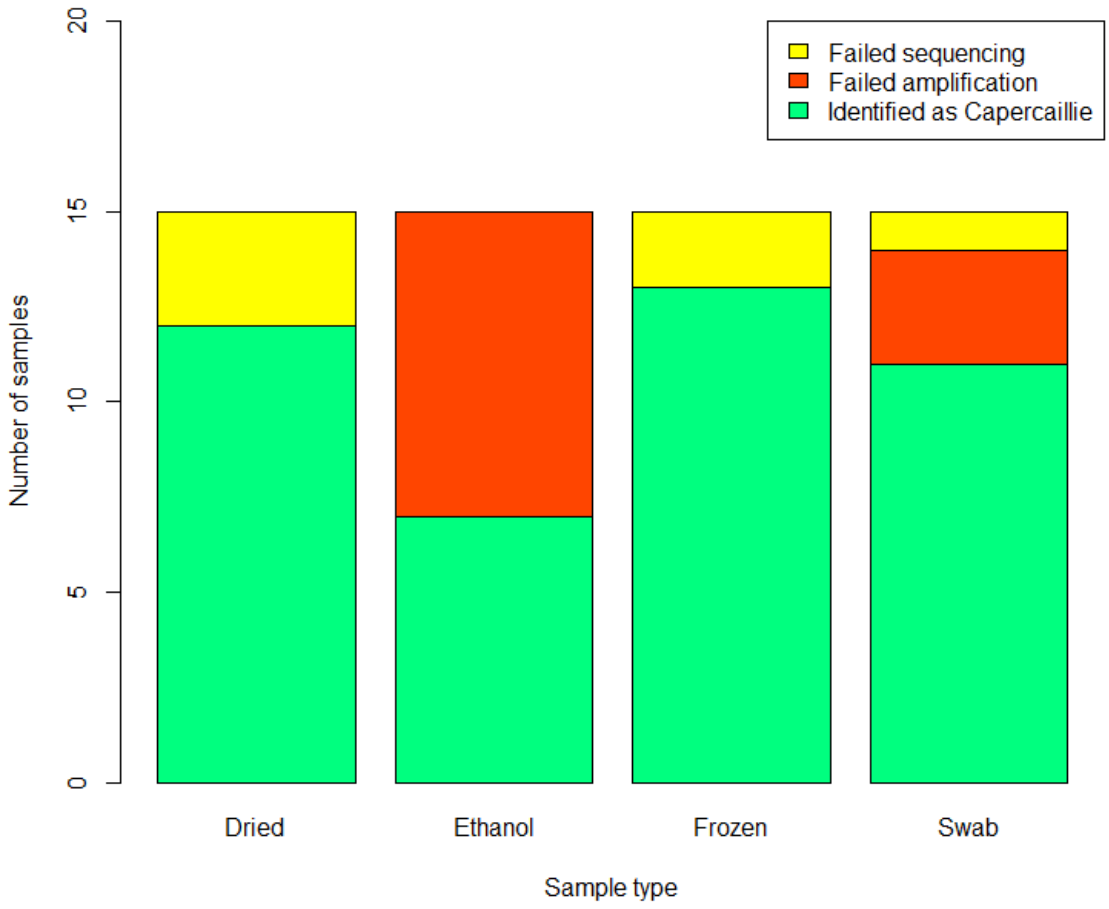

Figure 4. *A comparison of the number of samples that were genetically identified as capercaillie across the four different collection methods.*

C. Sex identification

Sexing has been attempted using the DNA extracts from all 60 samples. Using the Jensen et al. (2003) test we identified 31 samples as males. None of the samples were identified as originating from female capercaillie. The known female positive controls amplified as female in all tests but to double check the result in the absence of no female samples within the 60 tested we also tested 35 samples using a second avian sexing test (Lee *et al.* 2010). All 31 samples that amplified in the first test and 4 samples that showed very faint banding were tested with the second protocol. A total of 12 samples could be sexed using this second test, all of which were also identified as male. As sexing tests are reliant on high quality intact nuclear DNA, performing sexing tests allows us to identify the samples that are more likely to be useful for other downstream tests that are reliant on nuclear DNA. There were significant differences between sample success rate dependent on the collection method (Chi-squared test, $\chi^2 = 30.536$, df = 3, p-value = 1.065e-06). All the samples collected using the 'frozen' method could be sexed, however there were failures in samples collected via the other three methods (Figure 5). None of the samples collected via the 'ethanol' method could be sexed. A summary of results can be found in Appendix 1.

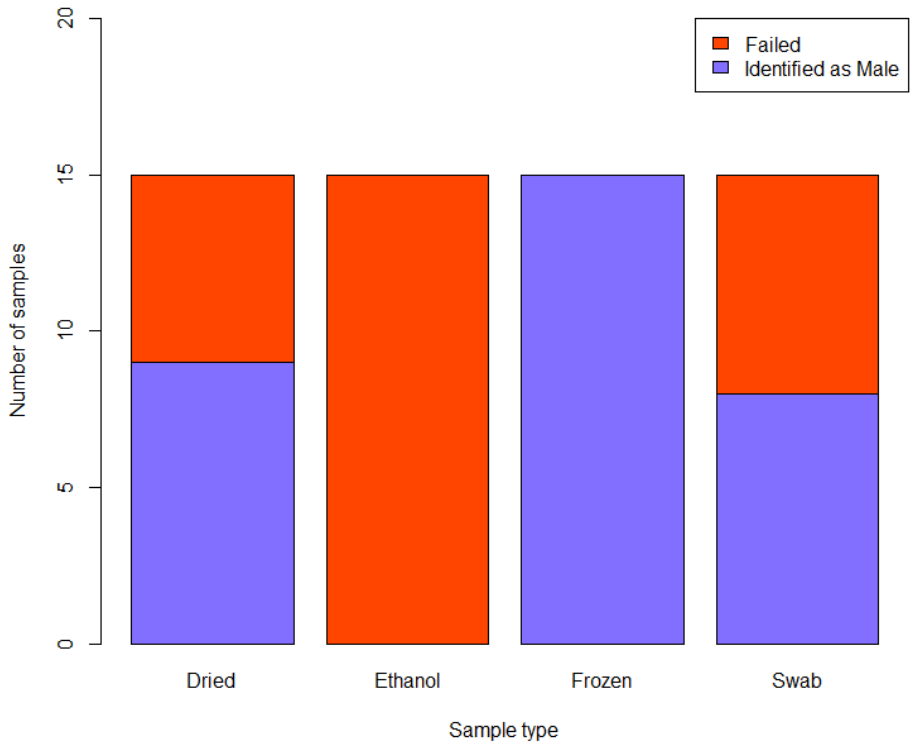

Figure 5. *Genetic sexing results of the faecal samples collected via the four differing protocols. All samples that could be sexed were identified as male.*

D. Microsatellite genotyping

The amplification and genotyping of 5 microsatellite markers, that have previously been used in previous capercaillie research via a multiplex PCR reaction, was attempted for DNA extracts from all 60 samples. Each sample was run in triplicate as DNA from faecal samples is often degraded and can contain inhibitors that can affect amplification and potentially cause allelic dropout. Consensus genotypes were created for each sample only if at least 2 of the triplicates were successful and the genotypes were identical. There was a significant difference between the genotyping success rate of samples collected via the four differing protocols (Figure 6, One-way ANOVA: df=3, F=10.35, p=1.61e-05). A Tukey multiple comparison test showed that the samples collected and stored via the 'frozen' method were more successful than those collected by the 'swab' or 'ethanol' protocols. The 'Dried' protocol was also more successful than the 'ethanol' method, which had no sample that produced a microsatellite genotype. Due to the potential for over dispersion in the dataset we also ran a robust generalized linear model in R using the 'robust' package. An analysis of deviance test showed that there was a significant effect of sample type on genotyping success (df=3, Deviance=49.71559 p=2.92e-10). The number of microsatellites that could be genotyped for each sample are summarised in Appendix 1 and the consensus genotypes for each sample can be found in Appendix 2.

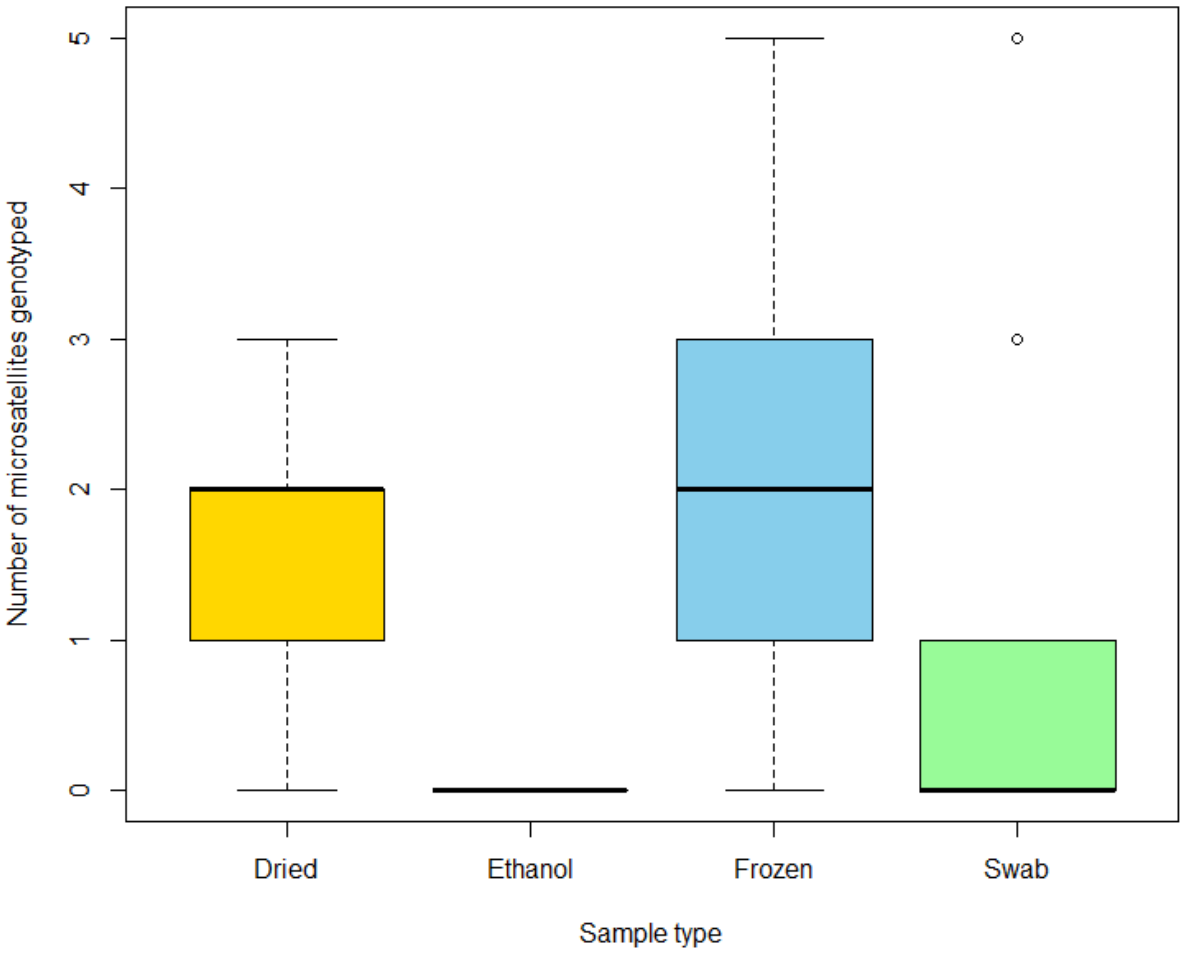

Figure 6. *A comparison of the number of microsatellites that were successfully genotyped within each of the four collection protocols. There were 15 samples collected via each method and genotyping was attempted on them all.*

ADVICE FOR FUTURE COLLECTIONS

The main result from this study shows that the samples collected via the 'frozen' method are the most suitable for capercaillie DNA preservation during future faecal surveys. However, the secondary result is that it is difficult to extract intact nuclear DNA from capercaillie faecal samples; only 2 samples (3%) successfully passed all three tests; being identified as capercaillie, sexed as male and genotyped at 5 microsatellite markers. However, there was clear variation between the 4 collection methods being tested, with the 'Frozen' and 'Dried' methods outperforming the other two. We advise that the 'frozen' method is used going forward and briefly summarise our findings for each method below.

Dried (with silica) sample collection

Samples collected via this method performed consistently well across the four comparisons. The highest quantity of DNA was extracted from these samples, however their slightly lower performance in the tests that required capercaillie DNA (compared to the 'frozen' samples) suggests the majority of this DNA is either highly degraded or not from capercaillie. Some of the dried faecal samples were the only samples to have fungus observed on the surface prior to DNA extraction in the lab. The areas with obvious fungus were avoided but the likelihood is that fungal DNA is a potential cause of the high amounts of DNA obtained during the extraction of these samples. However, capercaillie DNA was still present, as 80% (n=12) of the samples could be identified as capercaillie using mitochondrial DNA. A total of 60% (n=9) could be sexed and 20% (n=3) could be genotyped at ≥3 microsatellites. The sexing test provides evidence that nuclear DNA is present in over half of the samples.

Ethanol sample collection

This was the least successful method and we would advise it is not used for future capercaillie sample collection. On average, the lowest quantity of DNA was obtained from these samples. There is evidence that useable mitochondrial DNA is present in some of these samples as 47% (n=7) could be identified as capercaillie. However, we could not detect any useable nuclear DNA with every sample failing the sexing test and not a single microsatellite could be genotyped in these samples. One possible explanation for the low success rate of this method could be that it is the only one that does not target the external surface of the sample, which is where the epithelial cells from the capercaillie digestive tract are most likely to be present.

Frozen sample collection

This was the most successful method overall and is the method that we would advise is used going forward if capercaillie DNA is the target under investigation. On average, the second highest yields of DNA were obtained from samples collected via this method. However, compared to the 'dried' sample collection method this high DNA yield translated into higher success within all the tests dependent on capercaillie DNA. About 87% of samples were identified as capercaillie based on the presence of mitochondrial DNA. Critically, there was evidence of nuclear DNA in every sample, as 100% (n=15) of samples were sexed as male and 47% (n=7) of samples could be genotyped at ≥3 microsatellites. This method outperforms all others in the sexing test and performs marginally better than the second most successful method, 'dried', in the other tests reliant on capercaillie DNA. Importantly, one of the two samples that produced data in every test was collected via this method.

Swab sample collection

Samples collected via this method did not perform well and we would not advise that this current protocol is used for capercaillie going forward. This method showed inconsistencies between samples and between tests. Low quantities of DNA were initially extracted from the majority of samples, however there were a few outliers that produced yields more comparable to the 'frozen' samples. Despite the low yields, 73% of samples (n=11) contained capercaillie DNA, being correctly identified via the species ID test. There was also evidence of useable nuclear DNA with 60% (n=9) of samples identified as male and 13% (n=2) being genotyped at ≥3 microsatellites. Oddly, not all samples showing evidence of nuclear DNA showed evidence of mitochondrial DNA. Most surprisingly, one of the two samples that produced genetic data in every test, was collected via this method. One possible explanation is that this collection method relies on intricate swabbing in the field that could be highly affected by environmental conditions, collector effects or possibly its performance is more dependent on other factors (i.e. sample age or dietary components) compared to the other methods.

In order to maximise DNA quality and quantity for each sample we also include some guidelines below for continued collection:

A. General guidelines:

- During collection and storage, the samples should be touched as little as possible with bare hands in order to avoid contamination. Ideally each sample should be handled with a separate pair of disposable gloves. If these are not available, then using a nearby wooden stick or leaf to avoid touching the faecal sample.

B. Guidelines for sample collection:

- The most fresh faecal samples should be targeted for collection where possible, as older samples that have been exposed to UV and moisture for longer periods will have more degraded DNA and less chance of successful DNA analysis.
- In this study, 100% (n=43) of the identified samples were capercaillie. This suggests ID in the field is very good, however all samples that could be sexed were identified as males. This may not be surprising considering the samples were collected on a lek site dominated by males but it would be worth exploring whether potential differences in the faecal samples of females (e.g. smaller, different diet) could have caused them to be missed.
- When a faecal sample is collected it should always be stored in a separate container in order to decrease the risk of contamination by other samples.
- Make sure to label every sample collected with a unique identifier (e.g a letter code followed by a number and if space, with a date and location). Ideally a permanent marker is used for labelling both the top and sides of the sample container. However, if ethanol is used at any point we advise using pencil for labelling, as permanent marker can be dissolved during spillages.

C. Guidelines for sample storage:

- When back from the field, the samples should be placed in the correct storage conditions as quickly as possible. Based on the results of this study, the samples should be stored at -20°C as soon as possible and remain so until transfer to the lab.
- We suggest a container with a hard exterior is used for storage so that the samples are not damaged during transit or storage (e.g. a plastic falcon tube as used in the study here). A cotton plug can be used to limit movement of the sample if the container is too large.
- Check sample identifiers are on all tubes before placing them into storage.

These results suggest that faecal samples stored frozen at -20°C can be a useful source of capercaillie DNA for certain types of analysis and the WildGenes laboratory have found faecal samples to be a useful source of non-invasive DNA, as have multiple other studies (Broquet & Petit 2004, Segelbacher & Stenbruck 2001). However, the genotyping success of the microsatellite markers was low, only one 'frozen' sample (7%) could be genotyped at all markers. Faecal samples often contain PCR inhibiters especially if the species has a plant-based diet (Regnaut *et al*. 2006, Monteiro *et al.* 1997), and this can affect the usefulness of this sample type. Previous researchers have found DNA extraction for nuclear DNA work particularly difficult when working with capercaillie faecal samples (Segelbacher pers comm & Fletcher *et al*. 2018). However, there are studies that have found them useful (Jacob *et al.* 2010, Rosner *et al*. 2014, Aleix-Mata *et al.* 2019), the drawbacks of working with them could be compensated by increased sampling effort (with the knowledge that the majority of samples will fail), increased optimisation of lab protocols or the use of methods specially developed for degraded samples.

CONCLUSIONS

During this project the RZSS WildGenes laboratory have attempted to extract DNA from 60 faecal samples collected by the Cairngorms Capercaillie Project team. The samples were collected from leks and stored using 4 different methods, 15 samples per method. In brief, the methods consisted of storing the samples 1) Dried (in silica), 2) Frozen (at -20°C), 3) in ethanol (and frozen) and 4) via swabbing of the external surface (swab stored in a DNA preservation buffer). Species identification was possible for 43 samples (72%), all of which were identified as capercaillie. Sexing was possible for 33 samples (55%), all of which were identified as male. Microsatellite genotyping was possible at ≥3 of the 5 markers in 12 samples (20%). There was significant variation in the DNA quality between the collection methods, with the samples stored frozen at -20°C outperforming the other methods in all the tests that relied on the presence of capercaillie DNA.

A total of 13/15 (87%) samples stored frozen could be genetically identified as originating from capercaillie. Nuclear DNA could be extracted from all these samples, with 100% (n=15) being sexed as male. As with all collection methods the microsatellite genotyping was difficult to achieve but 47% (n=7) of the frozen samples could be genotyped at ≥3 microsatellites. Based on these results we advise that the 'frozen' collection method is used for any further collection of capercaillie faecal samples. However, if individual identification of samples is the aim of any further studies the low success rate of the microsatellite genotyping should be factored into decision making. To have enough power in the data to

distinguish individual genetic fingerprints and therefore identify samples originating from the same individual, the number of genetic markers needs to be maximised. The five microsatellite markers used here would not be enough for individual identification (and they could only all be genotyped in 2 samples) Ideally, tens of microsatellites or hundreds of single nucleotide polymorphisms (SNPs) would be required for individual identification (Waits *et al.* 2001). Due to the low success rate of microsatellite genotyping we therefore advise that either increased field effort is made (with the assumption that a majority of samples would not yield enough DNA) or an alternative method is used for genotyping, such as PCR-based SNP probes or a targeted enrichment approach for working with degraded samples is developed. A method currently being developed for degraded feather samples may be useful in regards to this latter point.

For any future sampling at lek sites it should also be noted that only male samples could be identified within this dataset. There are a variety of reasons why this may be the case, including 1) female faecal samples could not be genetically sexed, 2) Female faecal samples are not present (or are at very low levels) at the lek sites or 3) Female faecal samples at the lek site were not collected. We think that the first explanation is unlikely, considering that all the female positive controls were correctly identified and the genetic marker for the W chromosome is smaller and so less prone to degradation than the Z chromosome marker. If it is explanation 2 then surveys at lek sites may only be able to produce robust population estimates for males. If it is explanation 3 then perhaps field identification protocols need to be adapted so that samples from female capercaillie are also collected. The current field collection protocol was successful in identifying capercaillie samples as no evidence of other species were detected. However, the method used may have biased collection away from female capercaillie samples.

We recommend that all future collection of capercaillie faecal samples stores samples frozen at -20°C to maximise the capercaillie DNA obtained. The DNA obtained is still of variable quality but could be useful for a variety of questions. If individual identification is required for a mark-recapture population size estimation approach using microsatellites, it would be safe to assume that only about 30% of the samples collected by the 'frozen' method will produce useable genetic data. This is assuming the exact same collection protocol and microsatellite genotyping is used, further optimisation or an approach designed for degraded DNA is likely to improve this success rate. The main conclusion from this work is that capercaillie faecal collection with storage at -20°C has provided DNA most suitable for further genetic analysis. The quality and quantity of DNA obtained was highly variable between the 4 collection methods.

The work highlights the potential of faecal collection to be a useful technique for genetic monitoring of the capercaillie within the Cairngorms National Park if the most suitable collection method is used and there is an assumption that a proportion of the samples will not yield sufficient DNA. We advise that the use of genetic techniques designed specifically for use in degraded samples are pursued to increase this success rate.

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APPENDICES

Appendix 1. *Summary of results including a list of samples tested for this study, their respective DNA concentration evaluated using nanodrop (DNA conc. ng/µl), the species identified using a BLAST search, the results from both sexing analyses and the number of microsatellites genotyped.*

This can be found in the document entitled: **Appendix_1_Results_summary_for_faecal_samples.xlsx**

Appendix 2. *Details of the consensus microsatellite genotypes for each sample.*

The results can be found in the document entitled: **Appendix_2_CAP_concensus_msat_genotypes.xlsx**