

***FINAL Report:***



**Using genetic research to help secure the long-term future of  
the capercaillie in the UK**

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## CONTENTS

<b>EXECUTIVE SUMMARY</b> .....	3
<b>INTRODUCTION</b> .....	4
A. DNA extraction and quality control .....	6
B. Species identification .....	7
C. Sex identification .....	8
D. Biobanking .....	9
<b>RESULTS</b> .....	9
A. Sample quality .....	9
B. Species identification .....	11
C. Sexing .....	14
D. Biobanking .....	15
<b>ADVICE FOR FUTURE COLLECTIONS</b> .....	16
<b>CONCLUSIONS</b> .....	18
<b>REFERENCES</b> .....	19
<b>APPENDICES</b> .....	21

## 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\_003, awarded by the Cairngorms National Park Authority. The RZSS WildGenes laboratory was contracted for a period of 5 months (Nov 2019-March 2020) to analyse 800 feather samples collected by the Cairngorms Capercaillie Project. An additional 111 samples have subsequently been analysed. The main findings are outlined below:

1. DNA extraction was attempted from 911 feather samples and 1 capercaillie tissue sample.
2. The species for 751 (82%) of the feather samples has been identified.
3. Of the successfully extracted feather samples 663 (88%) were identified as capercaillie.
4. 18 other UK bird species were identified in the feather samples.
5. The sex of 383 capercaillie feathers have been successfully identified.
6. There are more male feathers within the collected capercaillie feathers (100 females:283 males).
7. We identify 383 feather samples for potential trial in a microsatellite analysis.
8. We have identified 663 samples for potential trial with hybrid-capture analysis.
9. DNA samples from 44 feathers have been identified that could be used for reduced representation genome sequencing (such as ddRAD) and are suitable for biobanking.

## 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 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 18<sup>th</sup> 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 2<sup>nd</sup> time in the UK.

The remaining populations of Scottish capercaillie are present in areas that are important for both wildlife and human activities. This leads to the potential for human-wildlife conflict and it is therefore critical to closely monitor the capercaillie populations through time. The current method used to estimate population size during the Scottish national survey is 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. The low number of individuals concentrated in one area also raises concerns regarding the future viability of the species in Scotland (Wilkinson *et al.* 2018). In this context, the monitoring of populations can help inform management decisions. 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; Gernot Segelbacher and Storch 2002). Moreover, establishing robust new techniques are likely to decrease the uncertainty currently associated with the population estimates.

This report outlines the initial feasibility of using a genetic approach for the population monitoring and management of capercaillie in the Cairngorms National Park by assessing feathers collected by the

Cairngorms Capercaillie Project team. The Royal Zoological Society of Scotland's WildGenes laboratory have been contracted to achieve the following:

- 1) Attempt to extract DNA from  $\geq 800$  feathers
- 2) Assess the quality of DNA obtained
- 3) Identify the species associated with each feather
- 4) Identify the highest quality samples for submission into a publicly visible and accessible biobank

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

## METHODS

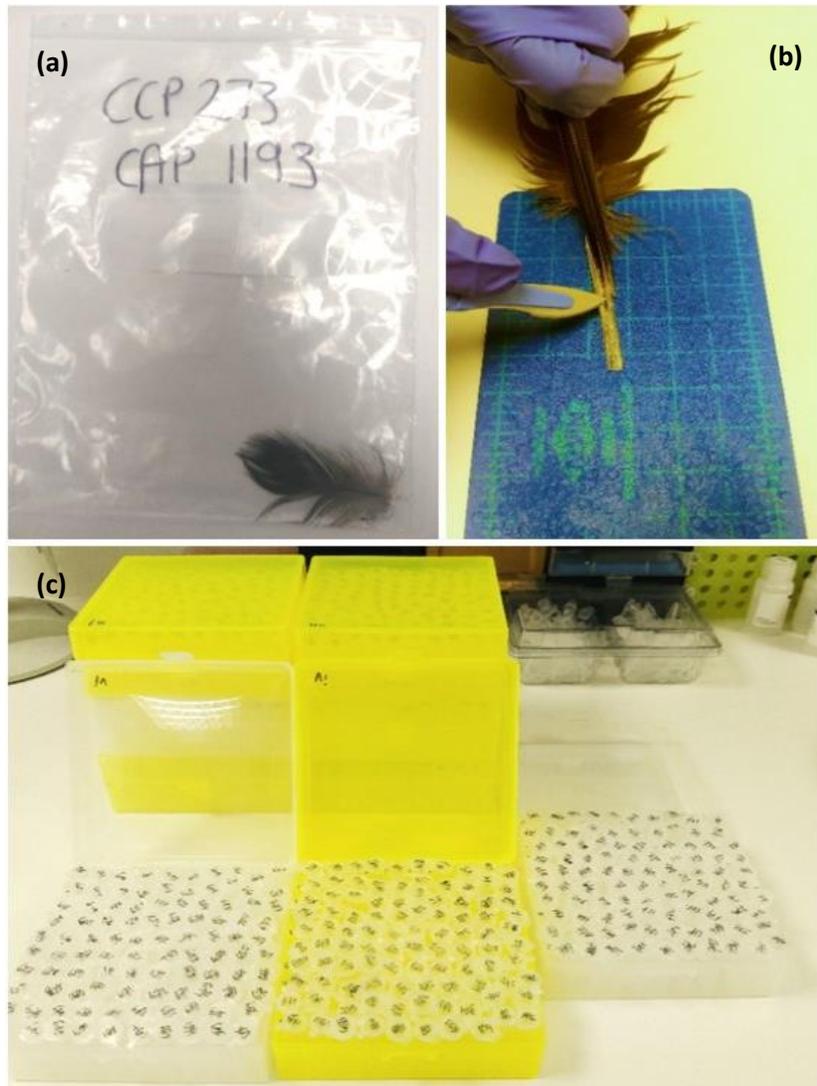
The RZSS WildGenes lab received a delivery in October 2019 of 1243 capercaillie feathers divided between 399 envelopes (Table 1). A single frozen tissue sample from a male capercaillie was also delivered. It was estimated that the feathers consisted of 907 unique samples as some envelopes were thought to contain feathers from multiple individuals.

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

Item	Count
Envelopes received	399
Total number of feathers received (data CNPA)	1243
Potential unique feather samples relabelled by RZSS	907
Additional control samples (4 feathers and 1 tissue sample)	5

Each feather was labelled with a unique identification number (CAPXXXX) and placed individually in a separate plastic bag for long term storage (Figure 1a). Once all the feathers had been sorted, a small part of the feather was used for DNA extraction. In most cases the feather was intact and the quill was used as a source of DNA. If a blood spot was present it was included in the extraction, as avian blood contains relatively high quantities of DNA. For large feathers the quill shaft was opened with a scalpel and the inside scraped in order to collect as much uncontaminated material as possible (Figure 1b). The entirety

of the calamus and the majority of the shaft was used for smaller feathers. All processed samples were placed separately in micro tubes before DNA extraction (Figure 1c).

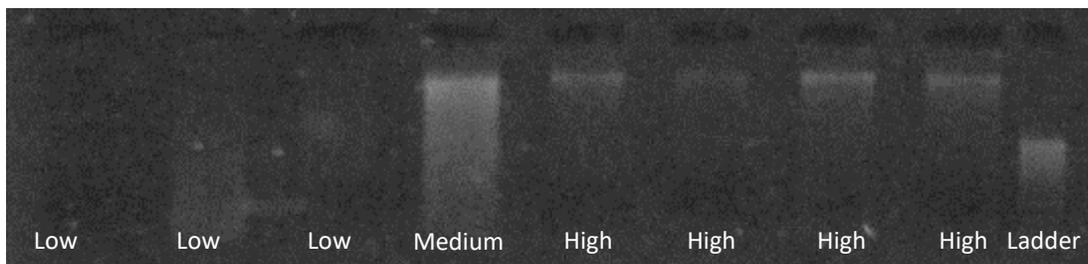


**Figure 1.** Feather samples being prepared for DNA extraction, (a) a feather labelled and prepared for long-term storage, (b) a large feather quill cut open and scraped in order to collect material for extraction, (c) samples stored before DNA extraction.

#### A. DNA extraction and quality control

In total, DNA has been extracted from 911 feather samples and 1 tissue sample (details in Appendix 1 and 2). All samples were extracted using the Qiagen Investigator kit following the manufacturer protocol with the exceptions of the addition of 5 $\mu$ l of DTT (1M) during the digestion phase, the digestion phase was also

modified to last overnight in order to maximise the final yield of extracted DNA. The amount of extracted DNA for each sample was then quantified using a Nanodrop Spectrophotometer (ThermoScientific). As non-invasive samples, such as feathers, contain low amounts of DNA they are more prone to potential contamination. In order to identify any contamination issues during the DNA extraction, a negative control was included for every 50 samples extracted (n=20 negative controls). After extraction, each DNA sample was run on an 1% agarose gel to check its quality. A good quality sample will have unfragmented, high molecular weight DNA. Electrophoresis on a gel reveals the size of the DNA fragments; a sample with high molecular weight DNA will fluoresce brightly and contain long DNA fragments that run more slowly through the gel. If extracted DNA is of high molecular weight it is more likely to amplify during a Polymerase Chain Reaction (PCR) and be easier to sequence, than DNA of low molecular weight (Figure 2).



**Figure 2.** Gel image of DNA samples for quality checking. The “Ladder” allows us to determine the samples molecular weight, samples categorised as “High” have high molecular weight, “Medium” samples have a faint smear indicating degraded DNA and samples are categorised as “Low” where no band can be seen.

## B. Species identification

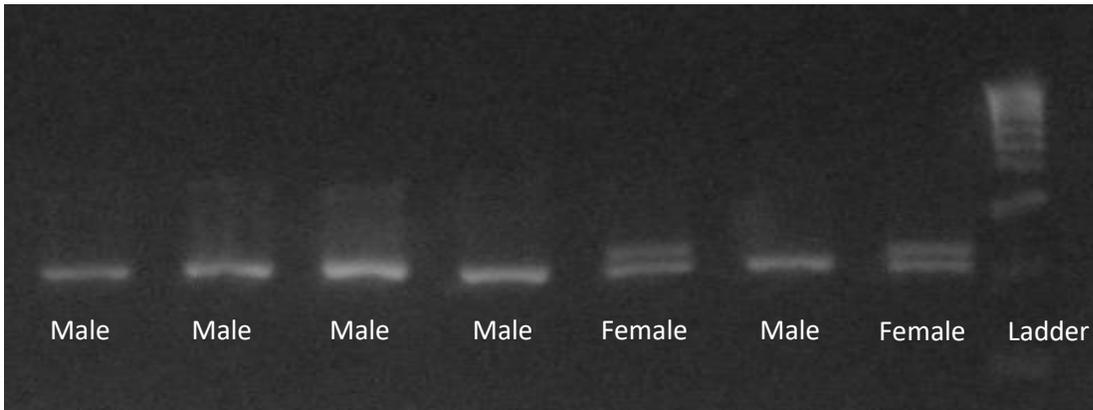
To identify the feather samples that originated from capercaillie, all samples were sequenced at a small region of the mitochondrial DNA (mtDNA) for species identification. A polymerase chain reaction (PCR) was used to amplify a region of the cytochrome B (CytB) mitochondrial gene using the previously 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. While this method is reliable for most samples, we also used an alternative method to check our species assignments; by constructing a phylogenetic tree we were able to verify the results from the BLAST analysis.

The phylogenetic tree was constructed within Geneious using the MrBayes package. The best substitution model and rate variation for building the phylogenetic tree were selected using the software R with the “modelTest” function from the phangorn R package. The model with the lowest AIC<sub>c</sub> was the substitution model “GTR” and the rate variation “invgamma”. It is important to include a distantly related species that you do not expect to find within the feather samples (termed an outgroup), we used ratites (including ostrich and kiwi) and mammals as the outgroups. It is important to note that this second method does not identify to species level for all samples, as it depends on the reference sequences that you use in its construction. However, it was able to identify all galliform (Gamebirds) samples to species level, as we included reference sequences for all wild galliform species found within Scotland. We also included a representative of all major bird groups found within the UK (see Appendix 3 for reference sequences). The phylogenetic tree allows us to identify which feather samples are capercaillie and if they are not, the most likely taxonomic group they belong to. Only the samples that were successfully identified as capercaillie by both the BLAST analysis and the phylogenetic tree were selected for sex identification.

### C. Sex identification

Sex identification was achieved using 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 published by Jensen *et al.* (2003). 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 3).



**Figure 3.** Gel image of a sexing test using the CHD-1 band. The males are identified by the presence of a single band and the female by the presence of two bands. The ladder is used to determine the molecular weight of each band.

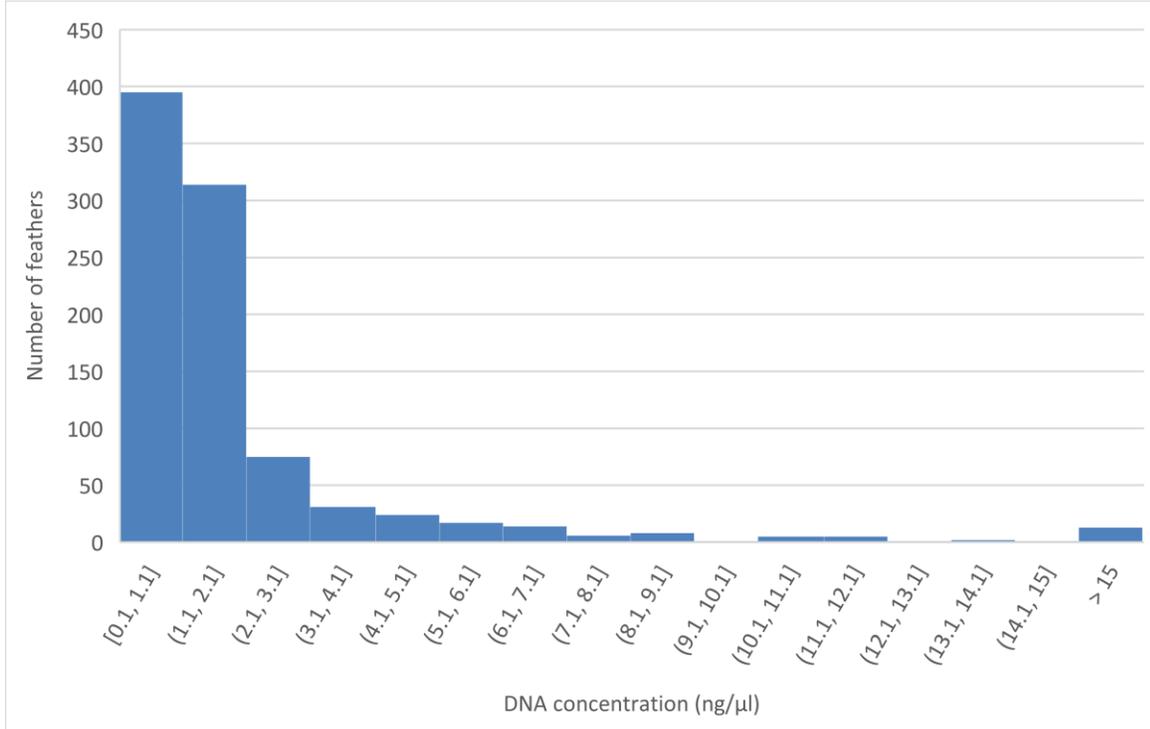
#### D. Biobanking

DNA samples have been handled with the objective of submission to the Cryoarks biobank initiative for long-term accessible storage (<https://www.cryoarks.org/>). This will allow them to be of use to the wider conservation research community. The extractions included negative controls, the freeze/thaw cycles have been recorded and the focal species have been identified with a high degree of certainty. Only samples identified as capercaillie and with large quantities of high molecular weight DNA will be entered into the biobank.

## RESULTS

### A. Sample quality

Of the 911 samples analysed, 82% (n=751) could be amplified using PCR and sequenced for species identification. The other 160 samples did not produce suitable fragments of DNA for sequencing and were discarded from further analysis. The quality control (QC) using agarose gels (Appendix 4) and the nanodrop showed that most extractions (n=727) produced a low quantity of DNA (<5ng/ $\mu$ l) that was not visible on a gel (see Figure 2 and Appendix 1). However, even though there was no visible DNA, small quantities can still be present within the extraction. This was confirmed by the nanodrop quantifications, which showed an average value of 2.4 ng/ $\mu$ l (Figure 4) and the success of the subsequent species ID tests.

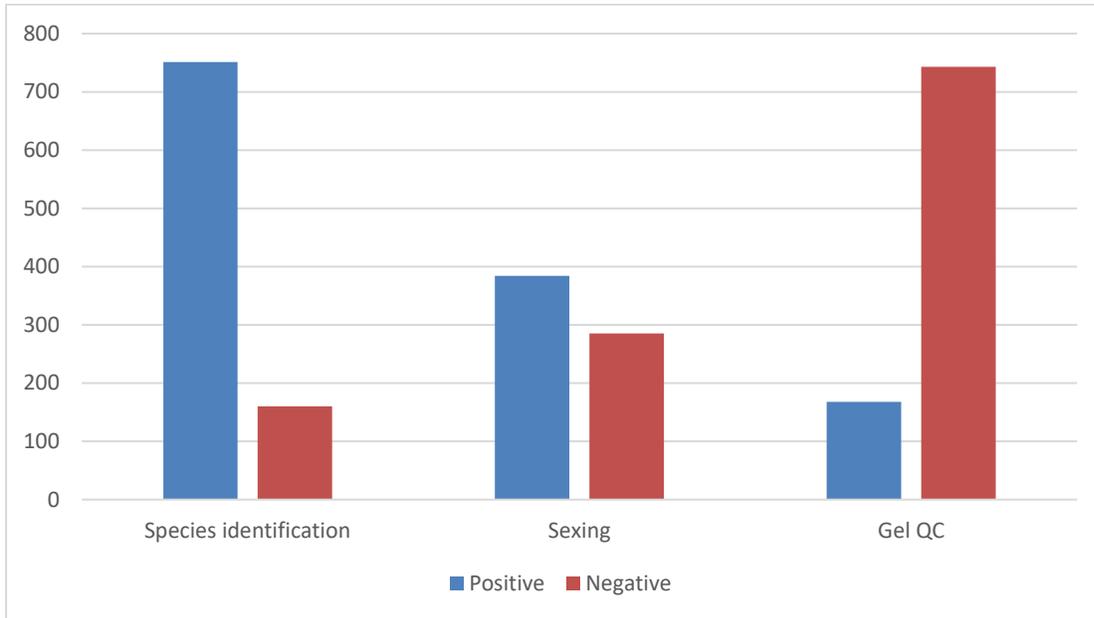


**Figure 4.** Bar chart representing the distribution of DNA concentration (ng/μl) for all feather extractions. Values obtained with a nanodrop spectrophotometer.

Of the 751 feather samples that it was possible to gain a species identification, 82% (n=618) showed no visible DNA on the QC gel. Of those that could be visualised on the gel, 28 samples were categorised as having medium quality (faint or smeared band) and 104 were categorised as high quality (clear band with high molecular weight). There were several samples (n=37) that were classified as medium or high quality based on the gel results but did not produce a species identification. This can occur if the DNA that has been extracted from the feather sample is bacterial or fungal, as it is possible to extract substantial amounts of good quality DNA, but that DNA is from bacteria or fungus present on the feather rather than from the animal that grew the feather.

Regarding the sexing analysis, 43% of the tested samples failed to amplify and could therefore not be sexed. Indeed, of the 668 feathers tested, 285 did not display any band or the band displayed could not be interpreted with certainty and were therefore categorised as failed. This result is not unexpected as the species identification test is based on mitochondrial DNA, however the sexing test requires intact nuclear DNA. Nuclear DNA (nDNA) degrades more rapidly than mitochondrial DNA and so in non-invasive

or degraded samples, tests requiring nDNA are going to be less successful than those relying on mtDNA. A summary of the results of the three procedures are presented in Figure 5.

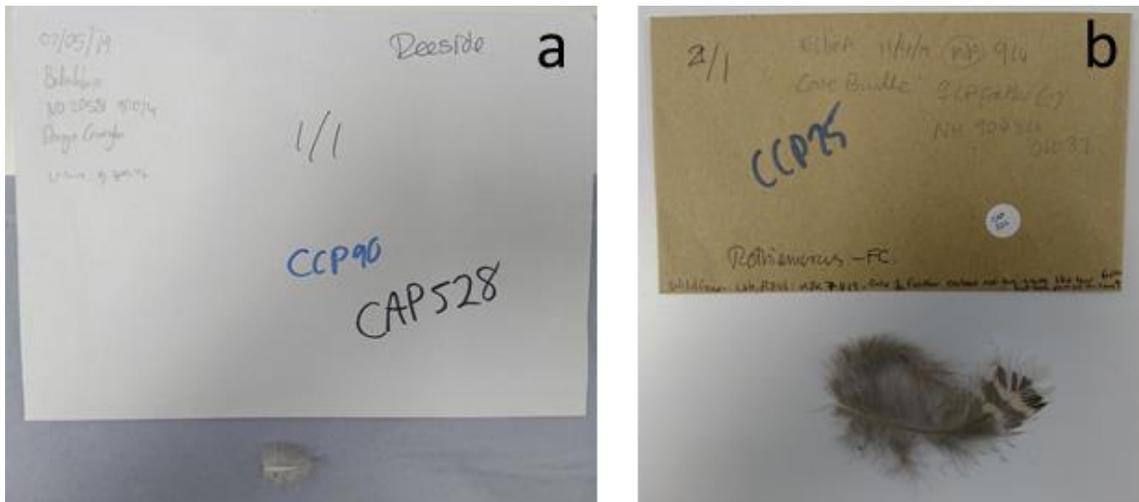


**Figure 5.** Bar chart representing the number of feather samples that produced a positive result in the three analyses conducted, next to the number that gave a negative result.

## B. Species identification

Species identification was attempted on all 911 feathers and successful sequences were obtained for 751 samples, of these, 663 (88%) were identified as capercaillie. The 88 remaining samples originate from 18 different bird species (Table 2). Two samples, CAP330 and CAP635, had a HQ above 80% and would therefore be considered as good sequences. However, they could not be included in the phylogenetic tree due to low quality base pairs at either end of the sequences. CAP330 matched capercaillie in the BLAST analysis but CAP635 did not match any species. Both of these samples were not included in further analysis. Also note that two feather samples were identified as mammals, one human and another as wild or domestic cat (CAP528 and CAP324 respectively). The species identification test we are using here cannot distinguish between the European wildcat and domestic cats, which originate from the near-eastern wildcat. These samples have obviously been contaminated at some stage, whether in the field, during collection, or in the laboratory. Humans are present during all stages and cats could also be present

in all locations, as the RZSS WildGenes laboratory does analyse wild and domestic cat samples during other projects. To investigate this further we first checked the negative extraction controls that were completed alongside these extractions and found there was no evidence of laboratory contamination. Furthermore, no wildcat samples had been used in the lab during the week that CAP324 was extracted. We also did visual checks of the two feathers, there were no obvious signs that they had been damaged. They are both small feathers which limits our ability to re-extract DNA (Figure 6). Although there is no way of ascertaining exactly where contamination has occurred, the above points suggest that contamination in the laboratory is unlikely. All other non-capercaillie feathers were identified as British birds.



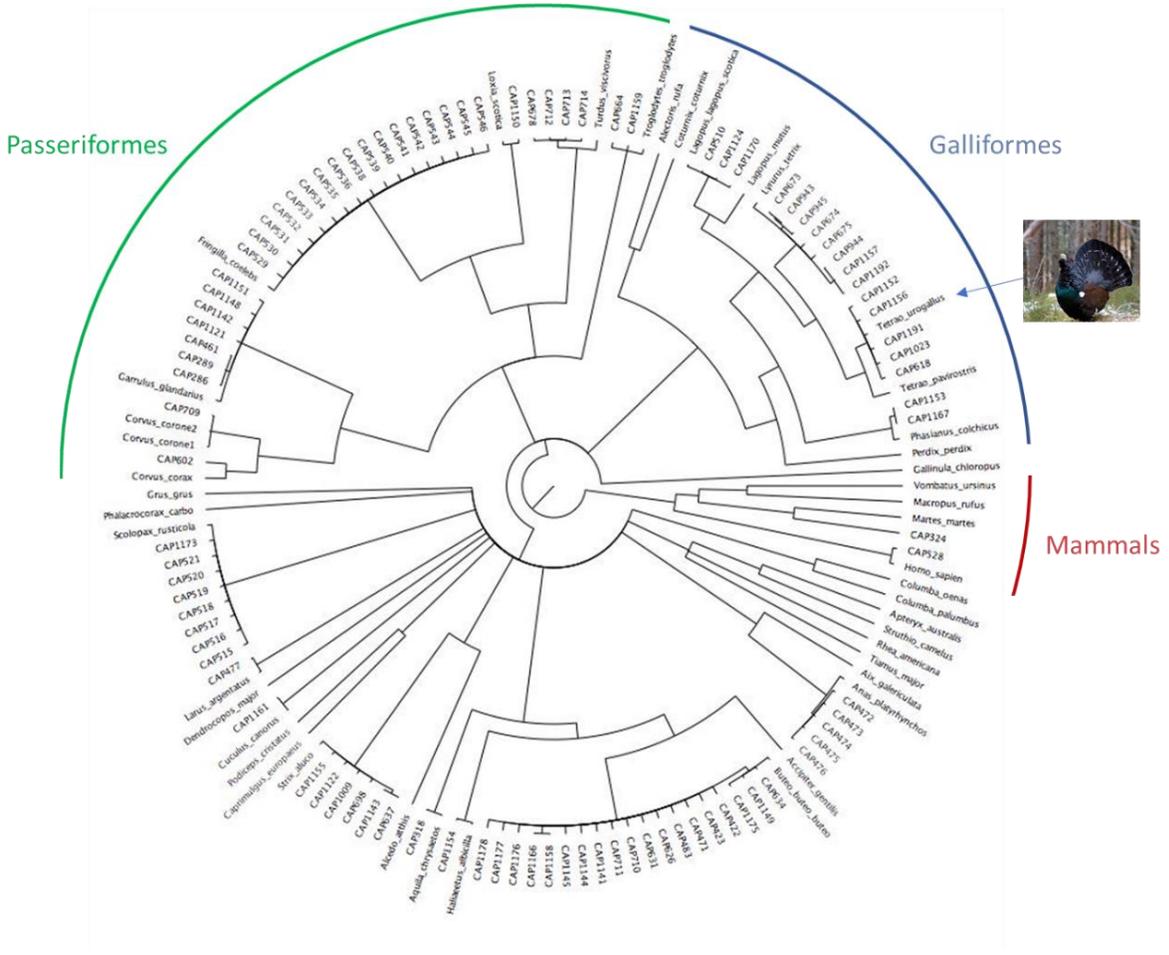
**Figure 6.** Photographs of the two feathers that had human (a -CAP528) and cat (b -CAP324) contamination in our species identification test and were thus excluded from further analysis.

The phylogenetic tree (Figure 7) was an additional method used to verify the results of the BLAST analysis. Phylogenetic trees are like family trees and are a way of visualising relationships between species. Closely related species are grouped together with short branches between them and more distantly related species have long branch lengths between each other. The 663 capercaillie sequences we identified all grouped together with the genetic reference sequence for capercaillie (NCBI Genbank number AB120132). By including representation species from the main bird groups found within the UK, any feathers that are not from capercaillie should group closer to one of the other species in the phylogeny. All results from the phylogeny supported the species identification obtained via the BLAST analysis (Appendix 5).

**Table 2.** List of species identified using a BLAST search of the NCBI database.

Species identified using a BLAST search (NCBI)	Species common name	Genbank accession number of top hit (in the case of multiple hits a single example is given)	Number of samples	Number of separate envelopes
Tetrao urogallus	Western capercaillie	AB120132	663	293
Anas platyrhynchos	Mallard duck	MK770342	5	1
Aquila chrysaetos	Golden eagle	Z73462	1	1
Buteo buteo buteo	Common buzzard	MK515312	19	17
Corvus corax	Common raven	AY527270	1	1
Corvus corone	Carrion crow	JQ864491	1	1
Cuculus canorus	Common cuckoo	AY274034	1	1
Fringilla coelebs	Common chaffinch	MN122830	17	1
Garrulus glandarius	Eurasian jay	EF602119	7	7
Haliaeetus albicilla	White-tailed eagle	X86753	1	1
Lagopus lagopus scotica	Red grouse	EF571187	3	3
Larus argentatus	European herring gull	EU526347	1	1
Loxia pytyopsittacus	Parrot crossbill	AF171664	1	1
Lyrurus tetrix	Black grouse	KF955638	8	5
Phasianus colchicus	Ring-necked pheasant	KF833639	2	2
Scolopax rusticola	Eurasian woodcock	HQ540480	8	2
Strix aluco	Tawny owl	MN122823	6	6
Troglodytes troglodytes	Eurasian wren	AY228084	2	2
Turdus viscivorus	Mistle thrush	EU154680	4	3
Felis catus/lybica/silvestris*	Domestic cat/wildcat*	KP202275	1	1
Homo sapiens*	Human*	MH449027	1	1
No data	NA	NA	158	105

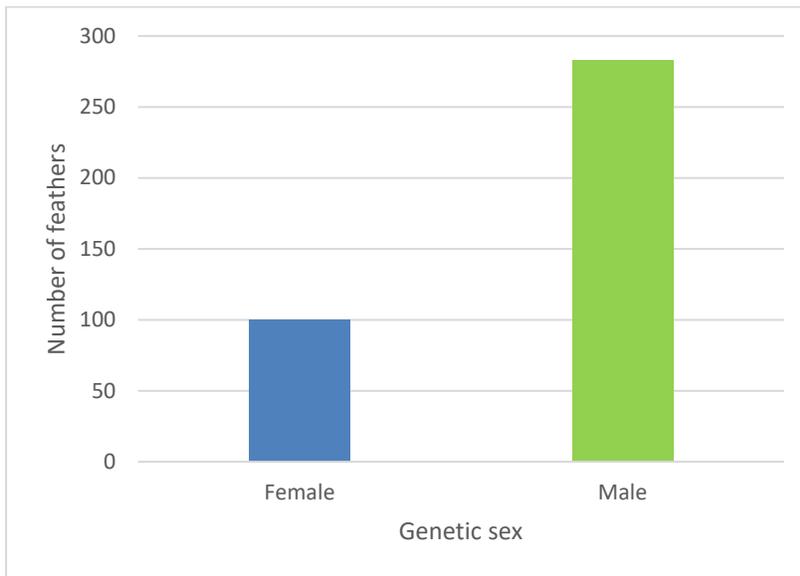
*Number of separate envelopes: some feathers arrived into the laboratory in the same envelope as others but were potentially from different individuals (as labelled by the CCP team) and so separated into individual sample bags by the WildGenes laboratory. \*contamination, see above for explanation*



**Figure 7.** Phylogenetic tree including several orders of bird species present in Scotland to identify the feather samples that were not from Western capercaillie (*Tetrao urogallus*). The tree was created using reference sequences of a 348 bp region of the mitochondrial gene, cytochrome B, and 95 sequences from the feather samples. Only 5 of the capercaillie sequences are included for visualisation purposes (see Appendix 1 for full results and Appendix 3 for a list of the reference sequences used).

### C. Sexing

Sexing has been attempted on 668 samples. Of these, the sex of 57% (n=383) could be identified, showing that 100 feathers were from female capercaillie and 283 feathers were from males (Figure 8). The sexing test had a high failure rate with 43% (n=285) of samples not providing results. This is a test that is routinely used by the avian genetics community and is used successfully on a regular basis in our lab. The most likely reason for this high failure is the low quality of DNA extracted from feather samples. All samples that were identified as capercaillie were tested.



**Figure 8.** Genetic sexing of the feathers that were identified as Capercaillie.

### D. Biobanking

Based on the testing of the 911 feather samples, 663 were identified as capercaillie, and 383 could be subsequently sexed using nuclear DNA. The gel QC shows that 44 of these samples have DNA of high molecular weight, making them suitable for next-generation sequencing (NGS) techniques. These samples would be valuable additions to a biobank, allowing them to be preserved in the long-term and making them widely accessible to the conservation research community. With the permission of the CNPA, the RZSS WildGenes lab has submitted these 44 DNA samples, plus the tissue sample (Appendix 6) to the Cryoarks initiative ([www.cryoarks.org](http://www.cryoarks.org)), a biobank database that is collating and preserving samples from museum, academic and zoological collections.

The results for each analysed feather are summarised in Appendix 1.

## ADVICE FOR FUTURE COLLECTIONS

The results from this study show that while the feathers can be useful non-invasive genetic samples, the majority do not contain the high quality and quantity of DNA that is often required for genetic techniques used to answer questions relevant to the conservation management of a species. We identified 44 samples that successfully amplified in a nuclear DNA sexing test and had high molecular weight DNA when visualised using gel electrophoresis. These are good indications that these samples could be used in future studies that rely on good quality nuclear DNA, such as microsatellites or Next Generation Sequencing techniques like ddRAD. Using these criteria, we submitted these 44 samples to the Cryoarks biobank initiative. The laboratory team attempted to maximise the quantity of DNA of all samples by using extraction techniques specially designed for samples with a low amount of DNA (Qiagen Investigator kit). This technique has been specially designed for and certified for use in forensic studies due to its ability to maximise DNA yield from degraded samples, but despite this it seems that many samples presented a low DNA concentration (Appendix 1). However, studies relying on mitochondrial DNA sequencing, such as the species identification test, will be able to use a greater proportion of the feather samples, a mtDNA sequence was successfully amplified in 82% of the samples. These results suggest that feathers can be a useful source of DNA for certain types of analysis and the WildGenes laboratory have found feathers to be a useful source of DNA, as have multiple other studies (Horváth et al. 2005; G. Segelbacher 2002; Vili et al. 2013). However, according to Vili et al. (2013), the quality of DNA obtained from feathers is impacted by humidity, direct sunlight and heat (in order of importance). It is therefore critical to avoid these factors during storage.

In order to maximise DNA quality and quantity for each sample and decrease laboratory costs we recommend the following guidelines:

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 avoid touching the feather shaft and instead pick up feathers by the end furthest from the quill tip.

B. Guidelines for sample collection:

- The feathers collected should be as fresh as possible, as old feathers that have been exposed to UV and moisture for long periods will have degraded DNA and less chance of successful DNA analysis.
- Feathers with blood spots or attached skin and tissue should be prioritised as they will have a much higher chance of successful DNA extraction.
- In this study, 12% (n=88) of the identified samples were not capercaillie. Although not very high, the number of non-capercaillie samples might be decreased by training the teams collecting the samples in capercaillie feather identification. The feathers might also be visually screened after collection and before they are sent for laboratory analysis. The results presented here could be used to compare the feathers from the non-capercaillie samples to the capercaillie feathers, to see if there are any distinguishing features. The WildGenes laboratory have stored the remaining feather fragments post-extraction that can be viewed/collected by the CCP team if deemed useful.
- When a feather is collected it should be stored in a separate container in order to decrease the risk of contamination by other samples.

C. Guidelines for sample storage:

- When back from the field, each feather should be dried at room temperature (it is important not to dry them using artificial heat as this can degrade DNA) away from natural light or UV light.
- Once each feather is completely dry (it is important that no moisture remains, as it has been shown to be one of the main causes of DNA degradation in feathers), it should be stored in a separate dry container, with silica if possible (silica absorbs moisture and there are silica beads that can indicate if the samples have been exposed to humidity during storage). We recommend that paper, precision wipes (e.g. Kimberly Clark precision wipes) or cotton wool be placed between the sample and the silica so they do not come into contact.
- Once dry the samples should be stored in a dry, cool and dark place. If there are risks of high humidity in the storage area, then once dry, samples can be stored in an air-tight container with the silica.
- Finally, if drying the feathers and storing them with silica is deemed too time consuming, it is also possible to freeze the samples. In order to do so, each sample should still be collected following the same guidelines, however there is no need to dry them or store them with silica gel. However,

it is extremely important to freeze the feather as soon as possible and that freeze/thaw cycles are avoided as much as possible.

## CONCLUSIONS

During this project the RZSS WildGenes laboratory have attempted to extract DNA from 911 feathers collected by the Cairngorms Capercaillie Project team. Species identification was possible for 751 feathers and sexing was possible for 383 samples (out of 663 samples identified as capercaillie). The results reveal that the majority (88%) of the feathers were from capercaillie and that more male than female feathers (283 males : 100 females) were collected.

Even though DNA was successfully extracted from a substantial proportion of the feathers, for the majority of feathers only very small quantities of degraded DNA were obtained. For future population genetic work on the capercaillie, larger amounts of good quality DNA would be preferable. Based on the results of the sexing test (which requires nuclear DNA) we estimate that 383 (42%) of the feather samples could produce scorable genotypes for population studies of capercaillie using a microsatellite panel. The degraded nature of non-invasive samples, such as feathers, would mean that this would need to be performed in triplicate to lower the risk posed by allelic dropout, which can lead to incorrect genotyping. Alternative techniques that provide much greater resolution to identify individuals and calculate genetic diversity are those based on next generation sequencing. However, these techniques require high quantity and quality of DNA and are rarely used on non-invasive or degraded samples. Recently, novel target enrichment techniques, such as hybrid capture, are increasingly being used to allow informative pieces of the nuclear genome to be amplified and sequenced from degraded samples. These techniques are revolutionising the use of non-invasive samples, by making it increasingly feasible to use them for an array of genomic techniques that produce extremely robust datasets for conservation management decisions. We advise that a hybrid-capture method would be the most suitable for future work with these non-invasive samples if considering long-term population monitoring and reliable management decision making.

We have made recommendations on feather collection and storage techniques that could increase DNA quality and hope that the species ID results presented in this report will help inform field identification techniques for future capercaillie feather collections. The main conclusion from this work is that capercaillie feather collection has provided DNA suitable for further genetic analysis. The quality and

quantity of DNA is highly variable between samples but there are some samples that provide DNA of sufficient quality to develop tools for future population management. This highlights the potential of feather collection to be a useful technique for genetic monitoring of the capercaillie within the Cairngorms National Park, especially if combined with DNA target enrichment techniques.

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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/ $\mu$ l), the species identified using a BLAST search (Species BLAST (NCBI), the results from the phylogenetic tree (Species phylogeny), the results from the sexing analysis (Sex), the results from the DNA quality analysis using gel electrophoreses (Gel quality) and the percentage of high quality base pairs in the DNA sequences calculated with Geneious (HQ% (Geneious)).

This can be found in the document entitled: **Appendix\_1\_Results\_summary\_for\_all\_samples.xlsx**

**Appendix 2.** Details of each extraction batch.

The results can be found in the document entitled: **Appendix\_2\_CAP\_extraction\_batch\_details.xlsx**

**Appendix 3.** Reference sequences used to create the phylogeny.

Genbank IDs of all reference sequences can be found here: **Appendix\_3\_Phylogeny\_Seqs.xlsx**

**Appendix 4.** Results from the sample quality test using gel electrophoresis.

The results can be found in the excel document entitled: **Appendix\_4\_CAP\_DNA\_Gel\_images.xlsx**

**Appendix 5.** Detailed results from the BLAST search (NCBI).

The results can be found in the excel document entitled: **Appendix\_5\_BLAST\_results\_NCBI.xlsx**

**Appendix 6.** Samples for the biobank

Details of the 28 samples submitted to Cryoarks: **Appendix\_6\_Biobank\_samples.xlsx**