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

Report on completion of sample collection

Date: July 2022

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Executive summary

This report constitutes the methods and findings from the collection of capercaillie droppings from lek sites as part of the Cairngorms Capercaillie Project. This includes reporting on the sample and storage trial and the genetic lek survey which will be used to compare genetic to traditional methods for counting birds at leks and therefore estimating populations. This report is designed to be read with the 2022 Royal Zoological Society of Scotland (RZSS) report 'Developing a new approach to estimating the size of the UK capercaillie population using genetic material'. A final report from RZSS is expected by July 2023, when the genetic analysis of samples collected at lek sites has been completed.

Two different sampling methods and four different storage methods (for droppings) were compared in spring 2021 to identify the optimum method for collecting capercaillie droppings to inform population estimates. Droppings were then collected during three separate visits to two capercaillie lek sites in spring 2022. The droppings were analysed by RZSS and the main findings are as follows:

1. Timed searches and freezing droppings were identified as the optimum sampling and storage methods respectively for collecting capercaillie droppings for population estimates.

2. There was variation in the number of droppings collected between sites and visits, which was thought to be impacted primarily by weather and capercaillie density and behaviour.

3. Most of the droppings were collected in close proximity to the lek centre at both sites.

4. It was more challenging to find high numbers of fresh droppings, with far more older droppings being available, which could be incorporated if the genetic analysis methods are able to obtain data from degraded samples (the final RZSS report will inform whether this is viable).

5. In this study, counting capercaillie using traditional methods took more time, but this varies significantly depending on the methods used. Volunteers can also be used for traditional methods, but this would be more challenging for genetic methods.

6. The genetic method is more expensive than traditional counting methods, so may be challenging to scale up to more leks.

7. There is the potential for the genetic method to be a less disturbing monitoring method, depending on the number of visits that are required to obtain samples, which will be informed by the final RZSS report.

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 second time in the UK unless more action is taken.

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 staff 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 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 estimates and can answer other questions of concern. Research has included using genetics to monitor population numbers (Rutkowski *et al*, 20171), investigate population dynamics (Bañuelos *et al*, 2019) and assess genetic diversity and health (Rutkowski *et al*, 2017).

In Scotland, two key capercaillie genetic studies include Piertney (2004) which assessed genetic diversity across Scottish metapopulations and Fletcher (*et al*, 2018); a pilot study to investigate whether genetic methods provide a feasible alternative field population monitoring methods applied in Scotland. 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 presents the methods used to collect capercaillie droppings from a sample of lek sites within the Cairngorms National Park, in order to test the viability of using these samples to estimate numbers of birds attending leks. This is split into two phases. The first reports on the methods and results from a sampling and storage trial completed in spring 2021, and the second phase reports on the results from the 'genetic lek survey' completed in spring 2022.

This report is designed to be read in tandem with the RZSS report 'Developing a new approach to estimating the size of the UK capercaillie population using genetic material' (2022). A final report will be produced by RZSS by July 2023, with the results from the genetic analysis of these dropping samples.

Sampling and storage trial

Background

In spring 2021, sampling and storage methods were tested to inform which methods should be used for the genetic lek survey. Previous genetic work in Scotland either didn't test droppings and used feathers instead (Piertney, 2004), or were unsuccessful in obtaining genetic information from droppings, as storage methods led to samples becoming too degraded (Fletcher *et al*, 2018). Therefore, a literature review was completed in 2020 (Doubleday, 2020), to review what sampling and storage methods were used in research that had successfully gathered population data from capercaillie droppings (outwith Scotland) to inform what methods should be tested.

Method

Sampling trial

The two most common methods from the literature that were used to collect capercaillie droppings were transects (Fletcher *et al* 2018 & Vallant *et al* 2018) and timed searches (Bañuelos *et al*, 2019), so these methods were compared as part of this trial.

In order to test the transect method, two lek sites were visited by the Capercaillie Advisory Officer in March 2021. One site has a low density of capercaillie (1 lekking male recorded in 2020) and the other had a higher density of capercaillie (14 lekking males recorded in 2020). At both these sites, 100m spaced transects were walked within 500m of the leks (last confirmed lek location in 2020) with sign and sightings of capercaillie recorded. The age of the droppings was also recorded, as only fresh samples would be collected for genetic analysis.

The results were compared to cold-searches of the same sites in March 2019 (when these sites were last cold-searched). Cold searches are used to inform early morning lek counts (usually completed from hides) e.g., by identifying hotspots of activity. During cold searches, transects aren't walked and surveyors are free to check all suitable habitat near the lek sites, which is a similar method to that used in timed searches.

The number/freshness of droppings and survey effort required were compared between these two sampling methods.

Storage trail

Capercaillie droppings were stored using a variety of different methods within the published literature. The different treatments selected to test during this trial included frozen, ethanol, dried (with silica) and swabbed. The latter treatment was informed by conversations with RZSS, as they have had success with this method for different species droppings.

For all treatments, capercaillie droppings were collected by the Capercaillie Advisory Officer and Assistant during cold searches completed between 10th March and 15th of April 2021. The aim was to collect 15 samples per treatment, focusing on collecting fresh droppings. These were collected as they were encountered during these surveys.

Frozen

Capercaillie droppings were transferred into 50ml falcon tubes, whilst taking care not to touch the dropping i.e., using plastic gloves, cocktail sticks, or another stick in the vicinity of droppings. These tubes were sealed with silicon tape and labelled with the date and unique sample ID number. Samples were transferred to a freezer on the same day of the survey.

Ethanol
Capercaillie droppings were transferred into 50ml falcon tubes, whilst taking care not to touch the dropping, using the same method as the frozen samples, until the tube was approximately half full (some samples needed to be cut to size). These tubes were then filled with ethanol (Analytical reagent grade, absolute 99.8%) up to the 50ml volume marker from a 500ml wash bottle with a squirty nozzle. These tubes were sealed with silicon tape and labelled with the date and unique sample ID number. Samples were transferred to a freezer on the same day of the survey.

Dried
The 50ml falcon tubes were prepared before the survey began, by filling the falcon tubes to approx. 15ml with self-indicating silica beads and placing a cotton pad on top, to act as a barrier to the sample. Capercaillie droppings were transferred into 50ml falcon tubes, whilst taking care not to touch the dropping, using the same method as the previous sampling methods. These tubes were sealed with silicon tape and labelled with the date and unique sample ID number.

Samples were stored in an airtight container and kept in a cool location away from direct sunlight. These samples were checked every 3 days and the silica was changed if it has started to change colour, which indicated that there was still moisture in the sample. Most samples required 3 changes of silica over a 2-week period.

Swab
When a fresh dropping was located, swabs (Isohelix) were soaked in 2ml tubes containing 1ml BuccalFix buffer solution, with excess solution removed by pulling the swab against the inner rim of the tube. The swab was used to lightly wipe the entire surface of the dropping twice over, rinsing the swab between, whilst avoiding touching the dropping. The swab was then placed into the tube of buffer solution, and this was stored in a cool location away from direct sunlight.

Results

Sampling trial
The results indicated that, although a wider area was covered using the transect method, far fewer samples (including fresh samples) were located using this method compared to the cold search method, which was consistent across both sites (Table 1 & 2). Therefore, timed searches were chosen as the method to proceed with for the genetic lek survey.

	Transects 2021	Cold search 2019
Number of droppings (piles)	21	89
Number of fresh droppings (piles)	3	40
Time spent searching (hrs)	5.25	4.5
Area covered (ha)	57	11.5
Birds seen	2 males (likely same bird)	1 female and 1 male

Table 1 – Results from both the transect sample method and cold search at the low density capercaillie forest

	Transects 2021	Cold search 2019
Number of droppings (piles)	42	115
Number of fresh droppings (piles)	8	61
Time spent searching (hrs)	6	6
Area covered (ha)	60	12
Birds seen	2 males	9 males (likely some the same)

Table 2 – Results from both the transect sample method and cold search at the high density capercaillie forest

Storage trial

Surveyors were able to collect 15 samples per storage treatment across 9 different forests. These were sent off to RZSS for analysis in September 2021. After analysis, the frozen method was recommended, as this was the most successful in obtaining the relevant DNA from samples (see full report 'Developing a new approach to estimating the size of the UK capercaillie population using genetic material', RZSS 2022).

Genetic lek survey

Background

After the sampling and storage methods were confirmed, a genetic lek survey was carried out in spring 2022. This had the aim of collecting droppings from leks, that could be analysed to identify numbers of birds attending leks, and compared to traditional lek count methods of observing birds.

Method

A sample of two leks were chosen from the Cairngorms National Park for this genetic lek survey. This included one high density lek (12 males recorded in the 2021 lek counts), hereafter referred to as 'Site 1' and one medium density lek (5 males recorded in the 2021 lek counts), hereafter referred to as 'Site 2'.

These leks were visited 3 times in spring 2022. The first collection for Site 1 was on the 14th of April, the second collection was on the 28th of April, and the third and final collection was the 5th of May. The first collection for Site 2 was on the 6th of April, the second collection was on the 27th of April, and the third and final collection was the 6th of May. These leks were also counted from hides on one visit during the last 2 weeks of April.

Samples were collected from a 500m circumference around the centre of the lek, which location had been recorded during the 2021 lek counts. Surveyors spent 4 hours searching for samples, starting from the centre of the lek and searching outwards, checking suitable habitat.

Only the freshest droppings were collected, and the same method was used as during the frozen treatment in the storage trial. Only one dropping was put in each tube. Surveyors used data recording sheets to record the site name, date, surveyor ID, grid reference (10 figure), sample ID code, sex of dropping, weather, and any additional comments.

Results

Number of droppings collected

There was variation in the number of droppings collected, both between sites and between visits (Table 3). At Site 1, a similar number of droppings were collected in the first and second visit, with more droppings being collected on the third visit. For Site 2, far fewer droppings were collected in the second and third visit, compared to the first visit. More droppings were collected overall at Site 1 compared to Site 2.

Most droppings were located in close proximity of the lek location. At Site 1, droppings were located in a plot size of approximately 8ha and at Site 2, droppings were located in a plot size of approximately 4ha. This does not reflect the area surveyed, rather where fresh droppings were sampled, although it was noted that surveyors didn't cover the entire 500m buffer during in any of these surveys.

Genetic compared to traditional lek counts

Each site took 4 hours to survey and approximately 1 hour of sample processing e.g., adding silicon tape to tubes and transporting them to a freezer. This means each site took 15 hours to survey with 3 visits.

Leks are generally counted once in the early morning. A lek count using a hide tends to start from 7pm the evening before and finishes when the birds stopped lekking the following morning, typically between 5-8. For these counts, Site 2 had a survey time of 11.5hrs (7pm-6.30am) with 2 surveyors, so an overall time of 23hrs. The survey time was not recorded for Site 1 (due to change in surveyors), but in previous years birds have been recorded lekking from approximately 4-9am. Therefore, it is likely that Site 1 had a survey time of 14hrs with 2 surveyors, so an overall time of 28hrs.

Site	Number of samples visit 1	Number of samples visit 2	Number of samples visit 3	Total number of samples
Site 1	34	31	44	109
Site 2	39	15	19	73

Table 3 – Number of capercaillie droppings collected from 3 visits to the 2 lek sites in spring 2022

Discussion

Sampling and storage trial

Both the timed searches and the frozen sampling method were deemed the simplest and most time-efficient methods.

Timed searches could more easily be incorporated within the existing survey work programme, as they are similar to cold searches, which are already completed. This method also took less time to complete. The transect method tested would have taken longer, but on each site, 3 transects were not completed, due to a combination of unsuitable habitat and time constraints.

Timed searches also recorded significantly more samples than the transect method. However, the 'freshness' of samples was fairly arbitrary and those recorded as 'fresh' during the 2019 cold searches may not be 'fresh' enough for genetic analysis, so this may have been overrepresented. Additionally, there was an unexpected drop in number of birds recorded at Site 1 during the 2021 lek count (6 males in 2021 compared to 14 in 2020), which may have contributed to less samples being located at this site in 2021.

The frozen method was also the quickest to complete, as sampling in the field was very straightforward and samples didn't need further processing once they were in a freezer. There were some practical issues encountered with the other storage methods. For instance, there was an issue with ethanol leaking when carried in the field, even with precautions in place to prevent this. The silica method required multiple changes of the silica, which took time to process. The swabbing method was challenging in the field, and it was difficult to use a consistent method between the samples, although storage of the swabbed solution was straightforward.

The frozen method was also the cheapest storage option, given that an existing freezer was available to use, whereas the other methods would require much more expensive materials, with ethanol being the most expensive, then swabbing then dried.

Genetic lek survey

Variation between visits & sites

The variation in fresh samples collected between site visits could be explained by the weather conditions, bird behaviour, or a combination of both.

The weather for the first and third sample collections for both sites was mild and fairly damp (i.e., rained night prior to collection and/or some light drizzle on day of collection), whereas the second collection, which resulted in the lowest number of samples at both sites, was far warmer and drier. This warm weather rapidly dried droppings, so fresh droppings were more difficult to identify.

Although the third visit resulted in the most droppings being sampled at Site 1, it took longer to find droppings with a wider area needing to be searched at both sites, suggesting birds were spending less time at the lek centre, which is to be expected as this is after the recognised peak time for lekking in Scotland. The third visit to Site 1 was influenced by the location of 2 fresh roosts, where multiple droppings could be sampled.

There was expected to be a variation between the sites, due to differences in the density of birds within these forests.

There was potentially an element of surveyor bias during these surveys, as the same surveyor was used at each site, and there was likely variation on what was deemed 'fresh droppings', as this assessment could be subjective and impacted by the weather.

Sampling methods

The 4-hour search time limit restricted the area that could be covered, indicated by the fact that the area within the 500m buffer wasn't completely surveyed on any visit. There is a chance that increasing the time limit could increase the amount of ground covered and therefore samples collected.

However, for Site 2, it was noted that far fewer droppings, especially fresh droppings, were located when a wider area was searched. This reflects the fact that there is only one lek in this forest, so the birds are less likely to use a wider area during this lekking period. Site 1 had more dispersed samples, with some fresh dropping being located at a greater distance from the lek, but there was a risk that a wider search could pick up birds from different leks, as there are multiple leks within this forest that regularly fluctuate in number.

There was uncertainty in how many droppings should be collected from the same location and what distance should be covered between sampling plots. Due to the fact that previous work in Scotland wasn't able to estimate populations due to low number of recaptures, and the results from the storage trial indicting low success in obtaining DNA, multiple samples were taken from each location, to increase chances of success.

There were far more older samples available that weren't collected at both these sites, which highlights that if older samples could be used, there would be a much higher sample size available. The new genetic analysis methods that will be used to analyse these droppings (i.e., Hybrid capture as opposed to the traditional approach of using microsatellites), will indicate whether it is possible to included older droppings for population estimates.

More visits to these lek sites would likely have yielded more samples, but these visits were restricted to reduce undue disturbance during a critical time for birds.

Genetic compared to traditional lek counts

Time

Although on this occasion, more time was required to complete a lek count compared to the genetic surveying, this can vary widely depending on the method and bird's behaviour.

For instance, some leks, generally those where the location isn't confirmed, are searched as an early morning walk-through, rather than using a hide. This reduces the survey time (as surveyors don't need to stay out the night before) and ranges from 2-5 hours, depending on the site. Birds also vary in how long they lek for, which can be impacted by the weather, number of males present (Summers *et al*, 2021) and presence of hens.

However, these calculations don't take into account the cold search time, as most leks are cold searched before the early morning lek count, which would add another 3-6 hours to the lek count total.

For the genetic method, it may be the case that a longer search time and/or more visits are required per site to gather enough samples for genetic analysis, in order to identify individuals, which will be informed by the final genetic report produced by RZSS.

Method & resource

Typically, counting leks using the traditional method requires a lower skill level than locating and identifying capercaillie droppings. This is particularly the case if surveyors are placed in hides and aren't required to search for capercaillie. This means that volunteers can and are used for lek counts, which may not be viable for this genetic method.

Additionally, the costs of completing lek counts using the traditional method are relatively low, as they utilise existing staff time and volunteers, with kit being restricted to a small number of hides and thermal imagers. Using genetic methods is far more expensive (approx. £220 per sample using Hybrid capture) even when using the lower cost storage option of freezing samples, so it's unlikely that this could be scaled up to cover a higher number of leks with existing resource.

Disturbance risk

Typically, using traditional survey methods, leks are visited twice during the spring. This includes a day time cold search, which is followed up with an early morning lek count. There are some occasions when these surveys are repeated, if resource allows and repeated effort is required e.g., a lek has moved location. The genetic lek survey required 3 visits, but these were all completed in the day, avoiding times when birds would be lekking.

It is difficult to conclude which method poses the highest risk of disturbance to the birds. The genetic survey has the potential to reduce disturbance risk, as it avoids the most sensitive time. However, the genetic survey should be restricted to the lowest number of visits as feasible and caution should be taken when deciding how many visits to complete, so this doesn't increase disturbance.

Conclusions

This work was able to identify the most accurate and time-efficient methods to collect capercaillie samples for genetic analysis in Scotland. The full results on whether genetic analysis successfully identified individuals, and is able to provide a total number of birds for each lek, will be informed by the final report produced by RZSS.

Recommendations

The following include some recommendations that should be considered if this genetic lek survey was to be repeated or expanded in Scotland.

1. Timed searches should be used as the sampling method, as they are the most time efficient and effective in locating samples. These should be completed by experienced capercaillie surveyors that are well adapted in locating capercaillie droppings.
2. The frozen method should be used to store samples, as this is the simplest and most time efficient method and was the most successful in yielding DNA.
3. A 500m buffer around a lek should be the maximum area covered to prevent sampling birds from other leks. If this entire area needs to be covered, then this would require more time and/or extra surveyors.
4. The method should be reviewed to confirm how many samples should be taken from each location and what distance should be walked between sampling plots, which will be informed by the results of the genetic analysis.
5. Different surveyors should be used during repeat visits at the same sites, to reduce the risk of surveyor bias.
6. More lek sites should be tested, including those that have even lower densities of birds e.g., 1 lekking male.
7. If this work was to be scaled up, it would likely require dedicated staff members to complete the fieldwork, as the staff involved were at capacity with 3 visits to 2 sites, which were completed alongside existing cold searches and lek counts.
8. Traditional lek counts should be continued alongside testing genetic methods, so that methods can continue to be compared.

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