Monitoring methods for the Golden Eagle *Aquila chrysaetos* in Norway

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ABSTRACT

Capsule: A description of the methods used for monitoring the Golden Eagle *Aquila chrysaetos* in Norway

Aims: To provide a comprehensive description of monitoring methods.

Methods: The intensive monitoring of the Golden Eagle in Norway started in 1991 as part of a national monitoring programme initiated by the Directorate for Nature Management (now the Norwegian Environment Agency). It has since become part of the Norwegian Large Predator Programme, and Golden Eagles are currently being monitored in 12 separate areas. Here we provide a comprehensive description of the current methods used in the intensive monitoring, with definitions, fieldwork and evaluation criteria for the final breeding status. In addition, a description of estimation of annual adult survival by genetic analysis is given. We describe the current methodology used in the intensive part of the Golden Eagle monitoring in Norway.

Results: We present some results derived from the Norwegian monitoring system and discuss the potential for further analyses. In addition, we highlight aspects in the monitoring of the Golden Eagles where our methods deviate slightly from those applied in other countries and the potential effects of these.

Conclusions: Intensive long-term monitoring programmes, such as this, will become increasingly valuable for monitoring the impact of environmental change, both from natural phenomena and from anthropogenic activities. To facilitate comparisons among the Golden Eagle monitoring programmes, detailed knowledge about the various methods applied is important.

Monitoring of raptor populations provides insight into the status of the populations and the factors that influence them (Hardey *et al.* 2013). Reproduction is a central parameter in population dynamics but in long-lived bird species with small clutch sizes, population dynamics is greatly influenced by adult survival (Steenhof & Newton 2007). In species like the Golden Eagle *Aquila chrysaetos* it is therefore important to monitor adult survival in addition to reproduction.

The Norwegian breeding population of the Golden Eagle has been estimated to be a minimum of 963 pairs (Dahl *et al.* 2015), but this estimate does not include estimates of pairs in areas with unknown presence. The species is classified as Least Concern on the Norwegian Red List (Henriksen & Hillmo 2015). A national monitoring database contains registrations from Golden Eagle breeding activities dating as far back as 1970. At present the Golden Eagle population in Norway is monitored through two different schemes, one extensive and the other intensive. The extensive monitoring covers most of the geographic

distribution of the Golden Eagle in Norway and data are collected by local conservation groups, local and regional management authorities and private persons. This monitoring scheme is not conducted in a regulated or organized way, thus collected data can only be used as observations of positive findings and not for detecting temporal or fine scale spatial variation in the Golden Eagle populations.

The intensive monitoring of Golden Eagles in Norway started in 1991 as part of the Monitoring Programme for Terrestrial Ecosystems (TOV); a national monitoring programme initiated by the Directorate for Nature Management (now the Norwegian Environment Agency). The most important objective of TOV was initially to monitor the flora and fauna in subalpine and alpine ecosystems to investigate impacts of long-range air pollution (Løbersli 1989). The objective was later broadened to include effects of climate change and responses to anthropogenic changes (Framstad 2017). The intensive monitoring of the Golden Eagle follows strict pre-defined protocols and methods to

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document both positive (i.e. breeding attempts) and negative findings (i.e. non-breeding). The monitoring in TOV was initially carried out in five areas with 10–13 territories in each area. From 1997 the monitoring was extended to six areas (Figure 1). In 2013, Rovdata, an independent unit within the Norwegian Institute for Nature Research (NINA), became responsible for the monitoring of the Golden Eagle in Norway as part of the Norwegian Large Predator Monitoring Programme (www.Rovdata.no). In Norway, the Golden Eagle is of management concern as it predates free-ranging livestock (Sheep *Ovis aries*; Mabille *et al.* 2015), and semi-domestic Reindeer *Rangifer tarandus;* Tveraa *et al.* 2014). Because the Golden Eagle is protected, the government pays compensation to livestock owners for killed livestock (documented and estimated losses).

Data on breeding success and adult survival to quantify population status and to understand fluctuations in population of the Golden Eagle in Norway are therefore crucial for management (Norwegian Environmental Agency 2015).

When implemented into the predator monitoring programme, the number of monitoring areas for the Golden Eagle was increased to 12 sites (11 sites in 2013 and 1 additional site in 2015; Figure 1) in accordance with a recommendation to the Norwegian Environment Agency (Gjershaug *et al.* 2012). This extension of areas allowed for an improved geographic coverage along both the north–south gradient and the east–west gradient of Norway to cover different environmental conditions present within the country. The intensive monitoring provides an estimate of
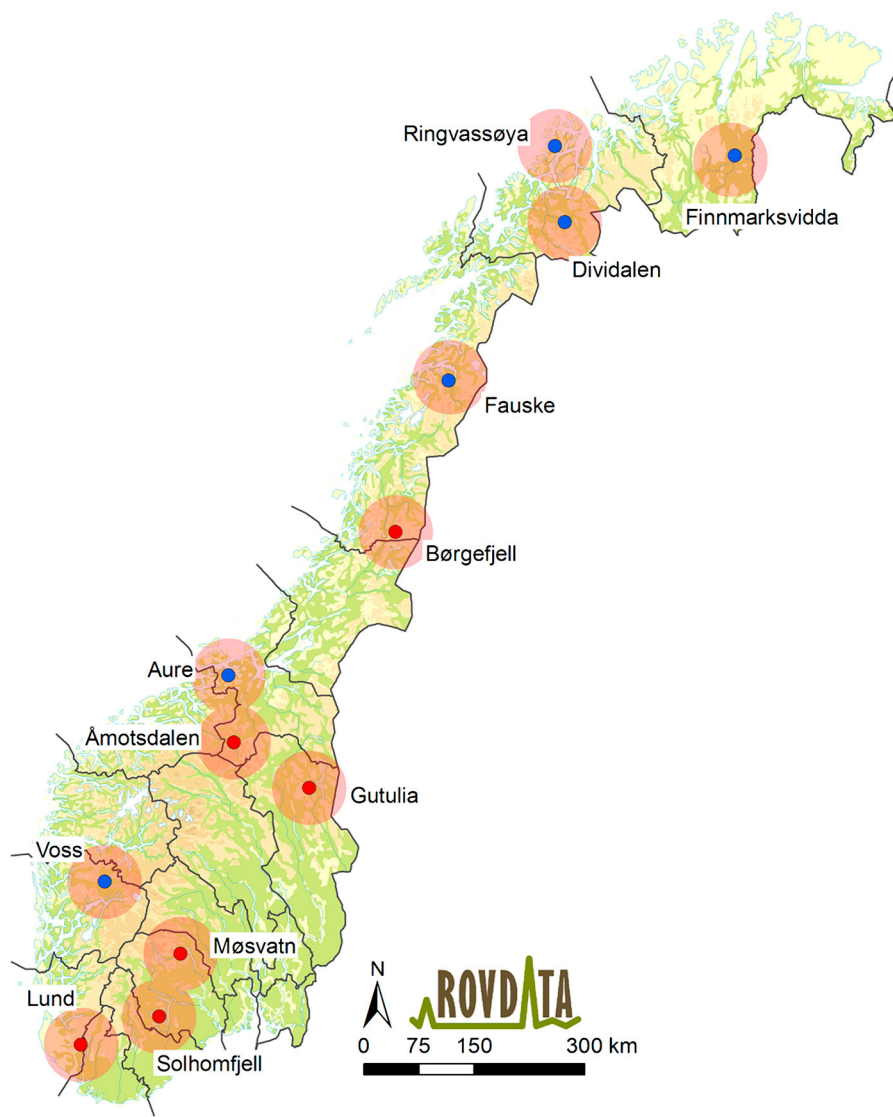

Figure 1. Intensive monitoring areas of Golden Eagle in Norway. The six TOV areas (Monitoring Programme for Terrestrial Ecosystems) in red have been monitored since 1991 (except *Gutulia* which was started in 1997) and those in blue have been monitored since 2013 (except *Aure* which was started in 2015).

breeding success defined by the mean number of fledglings for all monitored territories in each area which can be used to document both temporal population trends and variation between areas for this parameter. Today, 10–15% of the Norwegian population of the Golden Eagle is included in the intensive monitoring programme.

In this paper, we describe the protocol currently applied for the intensive monitoring of the Golden Eagle in Norway. The complete protocol, in Norwegian, can be found at http://www.rovdata.no/Kongeørn/instrukser.aspx. We also present some results to illustrate the kind of data the Norwegian intensive monitoring scheme generates.

Methods

Protocol for the intensive monitoring programme

Each of the 12 intensive monitoring areas contains 15 territories located within approximately 50 km radius from the centre point of the area. Each territory within the area is monitored as a separate unit. The intensive monitoring protocol of the Golden Eagle in Norway is based on a protocol previously developed for all the Nordic countries (Ekenstedt *et al.* 2006, Ekenstedt & Schneider 2008), including monitoring of the Golden Eagle within TOV in Norway. This protocol was slightly modified between 2013 and 2015 but only in a way which did not jeopardize the possibility of comparable data on breeding success with earlier data from TOV. The modifications were mainly related to more detailed demands for timing of the different field activities during the breeding season.

Definitions

Territory: The area used by a pair of eagles in the breeding season, which is defended against other pairs of eagles.
Nest site: Location of a nest.
Nesting area: Polygon, with 1 km buffer, around all known nests in the territory.
Occupied territory: The territory is defined as occupied when at least one of the following observations are made: (1) copulation, courtship feeding, incubation, eggs or nestlings; (2) two eagles (sub-adult or adult) observed together at least once in the nesting territory in the period 1st February–15th September; (3) one sub-adult or adult observed in the nesting territory several times in the period 1st February–15th September; (4) aggressive behaviour in the nesting territory; (5) flight display in the nesting territory or (6) nest supplied with fresh nest material.

Breeding attempt: Observations of incubation, eggs, feeding of nestlings, live or dead nestlings.
Cancelled breeding: The breeding is regarded as cancelled when the field observer has monitored all known nests, and the pair is observed together for a minimum of one hour without visiting a nest or showing behaviour related to parental care during the incubation period (15th April–10th May). It is often impossible to distinguish whether eggs were never laid (no breeding attempt) or if eggs are lost early in the incubation period (unsuccessful breeding).
Breeding success for an area: Average number of nestlings reaching over 49 days of age per 15 pre-selected territories. Not all territories would necessarily be classified as occupied each year.
Unsuccessful breeding: A breeding attempt is defined as unsuccessful if at least one of the following criteria are met: (1) no nestling(s) observed before 1st of July in a nest in which incubation has previously been observed, or within 100 days after a visit when incubation was not yet initiated in a nest in which incubation was observed at a later visit (100 days represent a dynamic date to adjust for variation in initiation of breeding. Nestlings are not expected to leave the nest within 100 days from initiation of incubation); (2) dead nestling(s) in the nest before 1st of July or within 100 days after a visit when incubation was not yet initiated; (3) egg remains in the nest before 1st of July or within 100 days after a visit when incubation was not yet initiated or (4) two dead nestlings, one dead nestling and one addled egg or two addled eggs independent of date.

Fieldwork

The fieldwork is divided among three periods; *spring*, *summer* and *autumn*. The main goal with the *spring* visit (1st February–15th June) is to find out if the territories are occupied or not, and to identify nests with breeding attempts. At least one visit should be in the period February–March, when all known alternative nest sites should be inspected from a safe distance to avoid disturbance in this sensitive period. If no breeding attempts are documented, the observation period in spring should be at least four hours in the territory in days with good weather conditions. To detect breeding attempts the period from egg laying to 15th May is the most favourable. If the spring observations indicate cancelled breeding, the summer fieldwork can be replaced by autumn fieldwork (see below). The main purpose of the *summer* fieldwork (15th June–31st July) is to quantify the number of nestlings over 49 days old in each nest. The age of nestlings is determined by the

colouration of plumage on the body and the head (Figure 2), according to Hoechlin (1976) and Peterson (1997). If the nestlings are younger than 50 days old, a later nest visit is needed to verify that they reach this age in order to conclude successful breeding. Nestlings normally leave the nest when they are about 70–80 days old (Watson 2010). Fieldwork in *autumn* (1st August–15th September) is obligatory if the status of the territory is unclear after finishing the summer visit (i.e. observations of neither unsuccessful nor successful breeding). The *autumn* fieldwork should be done on days with good weather conditions for eagle flying activity (days with some wind and no rain) to enable documentation of fledglings from potential missed breeding attempts (new nest sites). At least four hours of observations are required within the territory if no fledglings are observed.

Evaluating criteria of final breeding status

All field activities and observations are registered in a national database (www.rovbase.no), and each territory is given an annual breeding status (Figure 3). After each season, all entered data are quality controlled, evaluated and summarized by Rovdata before the results are published in annual reports (e.g. https://brage.bibsys.no/xmlui/handle/11250/2425793).

The final breeding status categories are:

Successful breeding: Observations of nestlings over 49 days old in the territory before 31st August or observations of fledglings together with an adult bird in the territory before 15th September.

Observed breeding attempt: Eggs have been laid, but there are no observations of nestlings over 49 days old. Includes both *unsuccessful breeding* and *unknown breeding success*.

Breeding attempt not observed: When the criteria for *occupied territory* is fulfilled, but no breeding attempt or successful breeding is documented.

Figure 2. A very rare case of a clutch of three Golden Eagle nestlings, about 50 days old. Photo by Jan Ove Gjershaug.

No breeding: Territory not occupied.

The goal is to collect complete data from all the 15 pre-selected territories in each area each year, but for cases where fieldwork is not carried out in accordance with the protocol, or the site has not been visited at all, the final status for territory will be *Uncertain breeding* and *Not controlled*, respectively.

Adult annual apparent survival

Adult survival has a substantial impact on population viability, particularly in species like the Golden Eagle
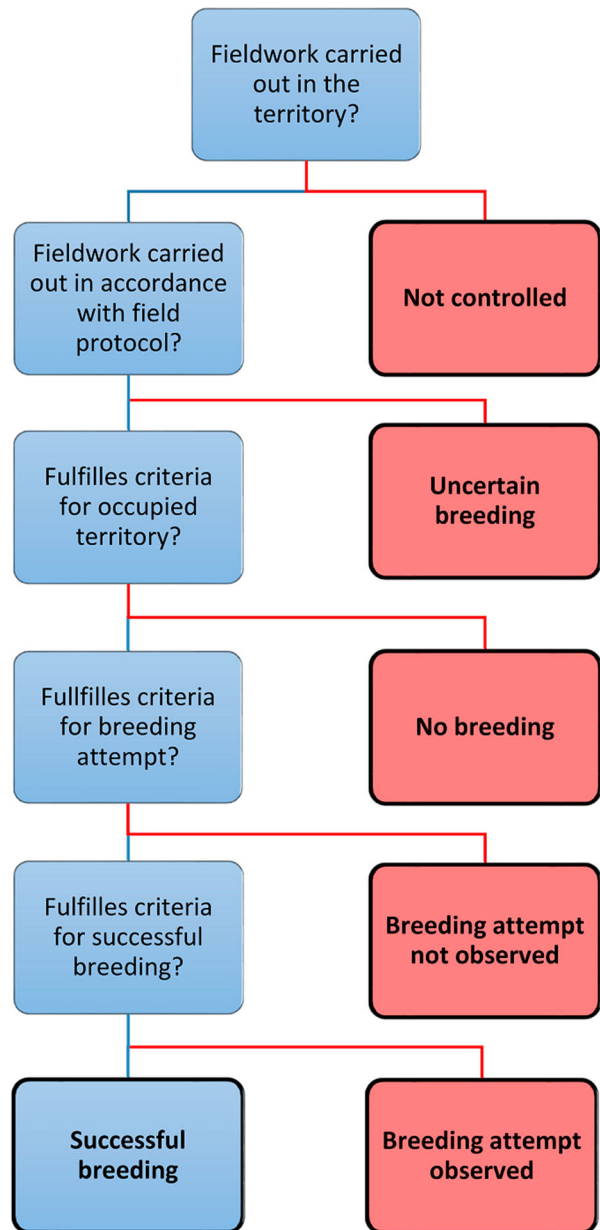

Figure 3. Procedure to evaluate the final breeding status (in bold). A positive answer to the question follows the blue lines (left column) while a negative answer follows the red lines (right column).

that is characterized by late maturation, long life span and small clutch size (Sæther & Bakke 2000). In addition to monitoring reproduction, a main aim of the programme is therefore to monitor adult survival. To examine the suitability of applying genetic monitoring to estimate annual apparent survival in adult Golden Eagles, sampling of moulted feathers and samples (pulled feathers or blood) from nestlings was initiated in 2012 in one (Finnmarksvidda) of the 12 intensive monitoring areas. Based on promising preliminary findings of the genetic monitoring in Finnmarksvidda, genetic monitoring was extended to also include a second area (Fauske) in 2015. The DNA-based methods applied to monitor individuals are identical for both areas, but the downstream analyses are based on data from Finnmarksvidda only.

Adult individuals are identified from a unique DNA profile through genetic analyses of non-invasively collected moulted feathers. As Golden Eagles are socially (and presumably genetically) monogamous and highly territorial, DNA analyses of moulted feathers and plucked feathers or blood samples from nestlings collected the subsequent year(s) can be used to identify territory owners and hence their annual apparent survival. For further information about the general principles of using genetic analyses to estimate annual apparent survival in raptors, see Rudnick et al. (2005).

Samples for DNA are collected in June and July as part of the summer fieldwork. Moulted feathers are collected from and underneath nest and roosting sites and, for nestlings, blood is sampled or developing feathers are plucked. Moulted feathers are stored in paper envelopes, the tip of nestling feathers is stored in 96% ethanol and blood samples are stored in lysis buffer at ambient temperature until analysis. Genomic DNA is extracted from feathers and blood using the Maxwell 16 tissue DNA Purification Kit following the manufacturer's protocol. Preferably we extract DNA from large moulted feathers in good physical condition (Vili et al. 2013) as such feathers provide the highest DNA quality yields (Hogan et al. 2008, Vili et al. 2013). The feathers are genotyped at 13 nuclear microsatellite loci and with a sex-typing marker (online Table S1). These loci were selected as they amplify relatively short fragments (<250 base-pairs), which likely increases genotyping success in moulted feathers in which the DNA can be degraded (Segelbacher 2002). Microsatellite loci are amplified using a polymerase chain reaction (PCR) with a Multiplex PCR *Plus* Kit (Qiagen) following the manufacturer's protocol, but using a 8.4 μL reaction volume. PCR products (0.8 μL) are mixed with GeneScan 500 LIZ (Applied Biosystems) size standard (0.14 μL) and Hi-Di

formamide (6.16 μL). Alleles are separated using capillary electrophoresis on an ABI 3130xl Genetic Analyzer and sizes assigned using GeneMapper software (Applied Biosystems). DNA from blood and plucked feathers are analysed in one PCR replicate. As the quantity and quality of DNA in moulted feathers can be low, DNA from each moulted feather is analysed in three (or more if required) independent PCR replicates. For each PCR a reference sample is included to control for fragment length scoring and a negative template control is added to control for false positive amplification and/or contamination. A consensus genotype is then constructed based on the following criteria: loci with a heterozygote result need to show this in two independent PCRs whereas loci with a homozygote result need to show this in three independent PCRs. Samples with a consensus genotype containing at least ten loci are used for individual identification. Unique genotypes are identified by using the program allelematch (Galpern et al. 2012). Capture-mark-recapture methods are used to estimate adult annual apparent survival.

Results

Here we give examples of the result on breeding success generated from the intensive monitoring by using data from two of the original monitoring areas, Børgefjell and Lund (Figure 1) for the period 1993–2016 (Figure 4). Both areas show a decreasing population trend and similar mean reproduction rate (0.52 ± 0.30 sd and 0.55 ± 0.18 sd), but one of the areas (Børgefjell) shows greater between-year fluctuations resulting in the negative trend being only significant for Lund (Lund: $r = -0.53$, $P = 0.007$, $n = 25$; Børgefjell: $r = -0.26$, $P = 0.22$, $n = 25$).

For the period 1993–2016 there was no significant correlation between reproductive rate for these two monitoring areas ($r = 0.20$, $P = 0.34$, $n = 25$). These areas represent different climatic conditions within Norway. Børgefjell is located in mountain habitat with cold winters and fluctuating onsets of spring and with unpredictable weather conditions, while Lund is located in southern Norway and includes more lowland forested habitats with a generally milder climate and more stable weather conditions in the breeding period. The areas are also likely to differ in the between-year variation in prey availability, with more pronounced 3–4 years cyclic fluctuations in rodent and small game (ptarmigan *Lagopus* spp. and Mountain Hare *Lepus timidus*) populations in the northern mountain area compared with the lowland area. In addition to a high variation in reproductive
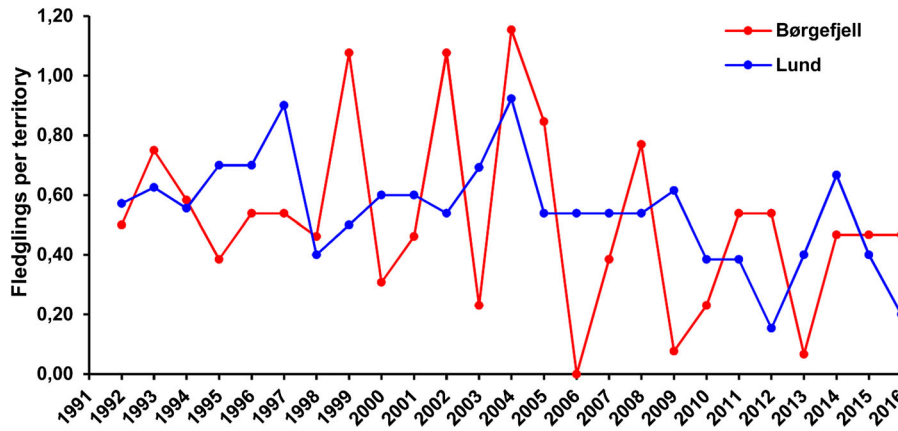
Figure 4. Reproductive rate (number of nestlings over 50 days old per territory) for two of the study areas: Børgefjell and Lund (see Figure 1).

rate between areas and between years, the monitoring also revealed that there was high variation in reproductive rate between territories within the same area. Using data from territories with at least 15 years of data, the best performing territories had an average

breeding success of 0.80 and 0.76 nestlings per year while the poorest had on average 0.22 and 0.40, in Børgefjell and Lund, respectively (Figure 5). Large differences in reproductive rate between territories are typical of many raptor species (Newton 1979), and are
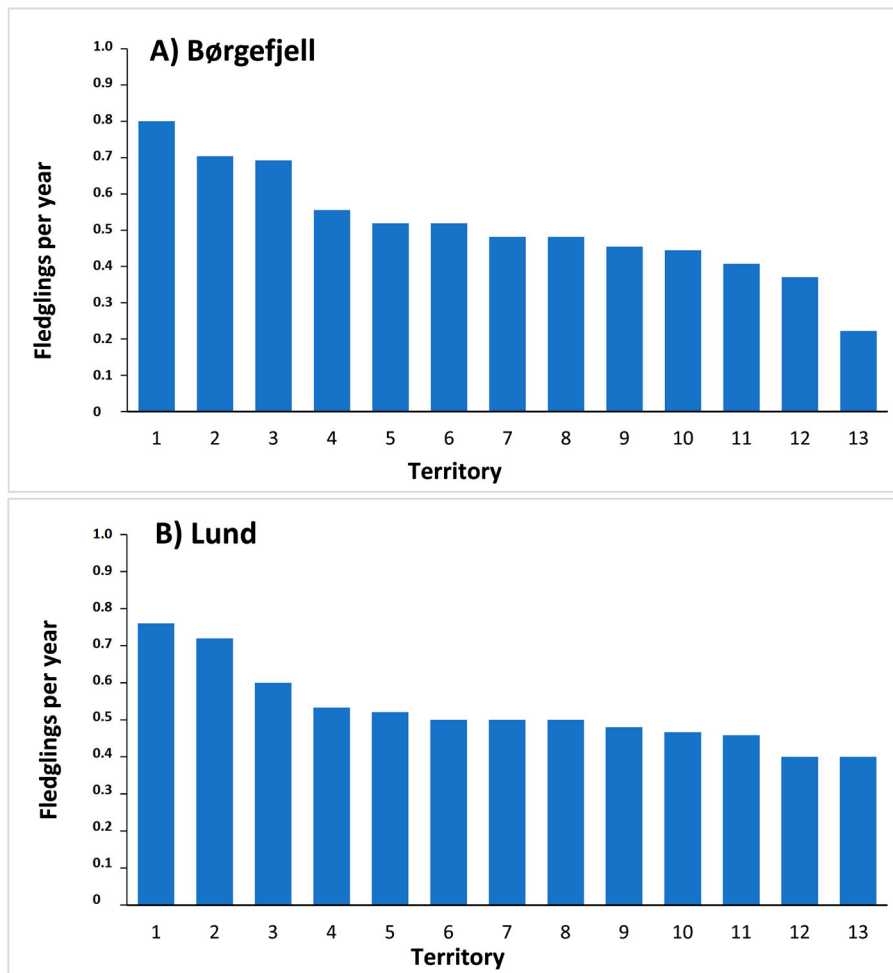

Figure 5. Variation in reproductive rate between 13 territories for the Børgefjell and Lund study areas in the period 1991–2016. Only territories with more than 15 years of data are included.
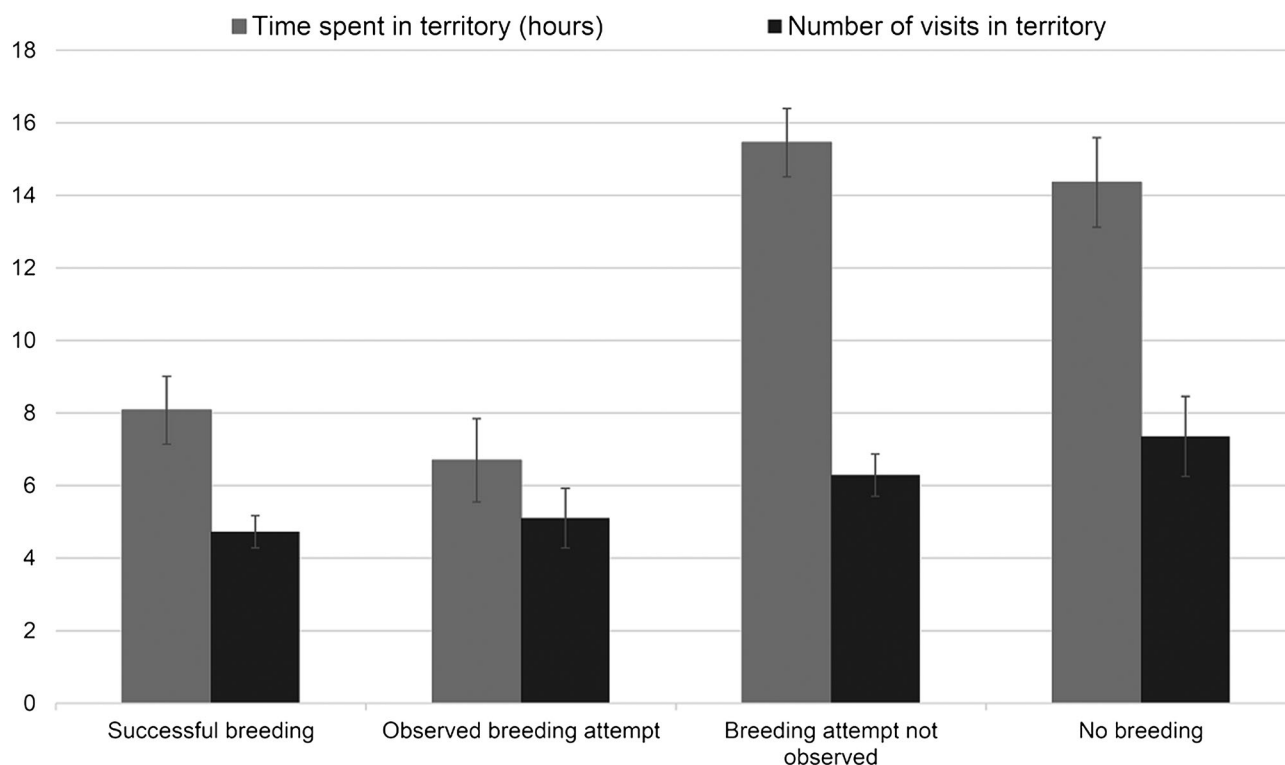
Figure 6. Time spent in each territory (grey bars) and number of visits per territory (black bars) in relation to the final breeding status, based on 415 surveyed territories between 2014 and 2016. Error bars represent 2 se.

probably caused by differences in the quality of the territories or the individual birds. The similarities and dissimilarities between the results (Figure 4) show the potential for future comprehensive analyses, including all areas in Norway, where the influence of different environmental and climate conditions on breeding success may be disentangled.

One of the major benefits with the intensive monitoring compared to the extensive monitoring is that each territory gets surveyed according to a detailed protocol. The extensive monitoring tends to be biased towards territory with positive findings (i.e. observers are more likely to return to an area where they have seen activity than where they have not). Looking at the intensive monitoring in Norway during the last three years, field personnel spent, on average, more time in territories without observation of breeding than where breeding attempts were documented (Figure 6). This is

necessary to identify eventual new nest sites and to increase the probability that a territory is finally classified as having no production.

DNA analysis

Genotyping of 36 presumably unrelated Golden Eagles from Finnmarksvidda revealed a mean of 6.6 alleles per locus (online Table S1). Observed heterozygosity ranged from 0.36 to 1.0 (Table S1). None of the 13 loci deviated significantly from Hardy-Weinberg equilibrium and no pair-wise locus combinations displayed significant linkage disequilibrium (Arlequin v3.5.1.2; Excoffier & Lischer 2010). Based on this set of 13 markers, the probability (p) of two individuals having an identical DNA profile by chance is low ($p = 2.5 \times 10^{-12}$).

Of 24 blood samples analysed from nestlings, all successfully provided a DNA profile. Of 191 moulted

Table 1. Adult Golden Eagles identified based on DNA analysis of moulted feathers and DNA profiles from nestlings in two of the monitored territories. Each individual is represented by a unique number (e.g. Ind0029).

Territory	Sex	Year				
		2012	2013	2014	2015	2016
A-NFI-076	Male	Ind0029	Ind0029			Ind0029
	Female	Ind0014	Ind0014	Ind0014		
A-NFI-118	Male		Ind0093		Ind0145	Ind0145
	Female		Ind0051		Ind0051	Ind0051

feathers that were analysed, 185 (97%) resulted in a DNA profile with at least 10 loci. These 185 feathers represented 39 individual Golden Eagles (20 females and 19 males). The presence of individuals varied across years as exemplified from two of the monitored territories (Table 1). The probability that an individual was encountered was estimated to be 0.74 (95% confidence intervals [CI] = 0.60–0.84) and annual apparent survival was estimated to 0.88 (95% CI = 0.77–0.94).

Discussion

Unbiased data on reproductive rates and survival allow comparisons among the Golden Eagle populations in different areas and different years and may reflect differences in land use, pollution levels, human activity or variations in natural phenomena such as weather and prey supply (Steenhof & Newton 2007). The methods used to quantify reproduction for the Golden Eagle presented here allow for comparison of yearly variation in number of fledglings caused by fluctuating environmental conditions and can detect both very good (breeding in all territories) and very bad years (few territories with breeding).

This method deviates slightly from those used in some other countries by including non-occupied territories (Hardey *et al.* 2013, Steenhof & Newton 2007). However, if territories that do not meet the criteria of being occupied are excluded, the breeding success may be overestimated especially in bad years. The different measures of breeding success can still be used for comparison if used with caution and with an awareness of the diverging criteria.

The topography of Norway creates great challenges when trying to determine if a territory is occupied or not, and would require a great deal of fieldwork effort which is often not possible due to economic limitations. The criterion 'flight display in the nesting area', to determine an occupied territory, may not always be reliable, as it is known that eagles from neighbouring pairs or unmated and non-territorial eagles can perform flight displays inside the core area of their neighbours' territories (Walker 2017). Data from the last two years of intensive monitoring in Norway show that 8–10% of the territories were classified as not occupied according to the criteria given in the methods. However, as discussed, this might be an overestimate. Because of these uncertainties all pre-selected territories, and not only those documented as 'occupied territories', are used in the calculation of breeding success. This alternative method is also described by Steenhof *et al.* (2017), and has been used by Kochert *et al.* (1999) and Steenhof

et al. (2014). We cannot see any disadvantages of this approach as far as the pre-selected territories are selected based on good information on historical use.

We regard nestlings aged over 49 days as the minimum requirement for a presumption of fledging in the Golden Eagle, as also recently recommended by Steenhof *et al.* (2017). This choice was taken to allow for ringing of nestlings while they are still small enough for handling. Some studies in Britain and North America have used at least 56 days (8 weeks) as the minimum age (Hardey *et al.* 2013, Steenhof & Kochert 1982). Our experiences suggest a very low mortality of nestlings once they are over 49 days old but are still in the nest. However, the exact age of nestlings to determine successful breeding is not that important as long as the same criteria is used (here ≥50 days) when data within a country are compared between areas and years. However, some caution should be used when comparing breeding success data between areas where different criteria have been used.

Here we have described our protocol for genetic monitoring of Golden Eagles in Norway. As shown in several other bird species, moulted feathers can provide a source of DNA with sufficient quality and quantity for genetic analysis to identify individuals (Rudnick *et al.* 2005, Bulut *et al.* 2016, Selås *et al.* 2017). By using the described method, we have successfully obtained a DNA profile in 92% of the moulted feathers analysed so far (unpubl. data). For bird species, like the Golden Eagle, that are difficult to trap and mark using traditional field techniques, DNA profiling of moulted feathers thus constitutes a powerful, non-invasive monitoring method that can be applied to obtain estimates on annual adult survival.

We expect that data from about 180 territories will have high statistical power to detect population changes over time. When the monitoring is based on a subset of the total population, the representativeness will always be questioned but our selected study areas are meant to provide a representative sample. Simulation models of adult survival show how the statistical power varies with the number of territories in the analysis. Based on data over 5 years from 30 territories, there would be an 80% probability of detecting a statistically significant change in survival as small as 10%. With a sample size of 15 territories, the probability of detecting such as small change would be 57% (Gjershaug *et al.* 2012). We have not done such calculations for reproductive success for this paper. We will point out that the aim of the monitoring is not to test hypotheses of reasons for differences between areas or periods, but to generate such hypotheses which later need more research to be tested.

Acknowledgements

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