Monitoring the Golden Eagle in Norway

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ABSTRACT

Capsule: A description of the methodology used for monitoring the Golden Eagle in Norway

Aims: To provide a comprehensive description of monitoring methods

Methods: We describe the current methodology used in the intensive part of the Golden Eagle monitoring in Norway.

Results: The intensive monitoring of the Golden Eagle in Norway started in 1991 as part of a national monitoring program initiated by the Directorate for Nature Management (now the Norwegian Environment Agency). It has since become part of the Norwegian Large Predator Program, and Golden Eagles are currently being monitored in 12 separate areas. Here we provide a comprehensive description of the methodology 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 DNA analysis is given. We discuss some aspects for monitoring Golden Eagles where our methodology deviate slightly from methodologies applied in other countries.

Conclusions:

Intensive long-term monitoring programs, 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 Golden Eagle monitoring programs, detailed knowledge about the various methods applied is important.

Keywords:

Aquila chrysaetos, Golden Eagle, monitoring, breeding success, breeding attempt, mortality, adult survival, DNA analysis

Monitoring of raptor populations provides insight into the status of the populations and the factors that influence them. In long-lived bird species with small clutch sizes, population dynamics is greatly influenced by adult survival (Steenhof & Newton 2007). Therefore, it is important to monitor adult survival in addition to reproduction. The total Norwegian breeding population has been estimated to 1225-1545 pairs (Heggøy & Øien 2014) and the Golden Eagle is classified as Least Concern (LC) on the Norwegian Red List (Henriksen & Hillmo 2015). A national monitoring database contains registrations from Golden Eagle activities dating as far back as 1970 and provide knowledge about the Golden Eagle in Norway. At present the Golden Eagle population in Norway is monitored through two different schemes, an extensive and an 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 fin scale spatial variation in Golden Eagle populations.

The intensive monitoring of Golden Eagles in Norway started in 1991 as part of the ´Monitoring Program for Terrestrial Ecosystems´ (TOV); a national monitoring program initiated by the Directorate for Nature Management (now the Norwegian Environment Agency). The most important objective of TOV was initially monitoring of flora and fauna in subalpine and alpine ecosystems to investigate impacts of long-range air pollution (Løbersli 1989). Later, the objective was broadened to include effects of climate change and response to anthropogenic changes (Framstad & Kålås 2016). The intensive monitoring of Golden Eagle, in contrast to the extensive, follow pre-defined protocols and methods to 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 to 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 Golden Eagles in Norway as a part of the Norwegian Large Predator Monitoring Program (www.Rovdata.no). In Norway, Golden Eagle is of management concern as it depredate on 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 economical 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 Golden Eagle in Norway is therefore crucial for management (Norwegian Environmental Agency 2015).

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When implementated into the predator monitoring program, 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 the recommendation from NINA 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. The intensive monitoring results in an estimation of breeding success defined by the mean number of fledglings for all monitored territories in each area which can be used to document both temporal trends and variation between areas for this parameter. Today, between 10 and 15 % of the Norwegian population of the Golden Eagle is included in the intensive monitoring program.

In this paper, we describe the protocol currently applied for the intensive monitoring of the Golden Eagle in Norway. An instruction in Norwegian language can be found at http://www.rovdata.no/Kongeørn/instrukser.aspx.

RESULTS

Protocol for the intensive monitoring program

Each of the 12 intensive monitoring areas contain 15 territories located within approximately 50 km radius from the center point of the area. Each territory within the area is monitored as a separate unit. The intensive monitoring protocol of 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 has been slightly modified between 2013-2015 but only in a way which does not jeopardize the possibility of comparable data on breeding success with earlier data from TOV. The modifications have mainly been related to more detailed demands to 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:

- copulation, feeding, incubation, eggs or chicks

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- two eagles (sub-adult or adult) observed together at least once in the nesting area in the period February 1st – September 15th
- one sub-adult or adult observed in the nesting area several times in the period February 1st – September 15th
- aggressive behavior in the nesting area
- flight display in the nesting area
- nest supplied with fresh nest material

Breeding attempt: observations of incubation, eggs, feeding of chicks, live or dead chicks.

***Cancelled breeding*:** The breeding is regarded as cancelled when the field observer has controlled all known nests, and the pair is observed together for a minimum of one hour without visiting a nest and showing behavior related to egg/chick, during the incubation period (April 15th – May 10th). 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 chicks ≥ 50 days per 15 preselected territories (all of them not necessarily classified as occupied each year).

***Unsuccessful breeding*:** A breeding attempt is defined as unsuccessful if at least one of the following criteria are met:

- no chick(s) observed before 1st of July in a nest in which incubation has previously been observed, or within 100 days[1] after a visit when incubation was not yet initiated in a nest in which incubation was observed at a later visit.
- dead chick(s) in the nest before 1st of July or within 100 days after a visit when incubation was not yet initiated.
- egg remains in the nest before 1st of July or within 100 days after a visit when incubation was not yet initiated
- two dead chicks, one dead chick 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 (February 1st – June 15th) 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

[1] 100 days represent a dynamic date to adjust for variation in initiation of breeding. Chicks are not expected to leave the nest within 100 days from initation of incubation.

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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 condition. To detect breeding attempts the period from egg laying to May 15th is the most favorable. If the spring observations conclude cancelled breeding, the summer fieldwork can be replaced by autumn fieldwork (see below). The main purpose of the *summer* fieldwork (June 15th – July 31st) is to quantify the number of chicks ≥ 50 days old in each nest. Age of chicks are determined by the coloration of plumage on the body and the head (Figure 2), according to Hoechlin (1976) and Peterson (1997). If the chicks are less than 50 days old, one need to return later to verify that they reach this age in order to conclude successful breeding. Chicks normally leave the nest when they are about 70-80 days old (Watson 2010). Field work in *autumn* (August 1st –September 15th) 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 activities (days with some wind and no rain) to enable documentation of fledged chicks from potential missed breeding attempts (new nest sites). At least four hours of observations are required within the territory if no fledged chicks 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. After each season, all entered data is quality controlled, evaluated and summarized by Rovdata (see introduction) before the results are published in annual reports (e.g. https://brage.bibsys.no/xmlui/handle/11250/2425793).

The final breeding statuses are:

Successful breeding: Observations of chicks ≥ 50 days in the territory before August 31st or observations of fledged chicks together with an adult bird in the territory before September 15th.

Observed breeding attempt: Eggs have been laid, but no observations of chicks ≥ 50 days. 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.

No breeding: Territory not occupied.

The goal is to collect complete data from all territories 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.

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Annual adult survival

Adult survival have great impact on population viability, particularly in species like the Golden Eagle that is characterized by late maturation, long life span and small clutch size (Sæther & Bakke 2000). In addition to monitor reproduction, a main aim of the Golden Eagle monitoring program is therefore to monitor adult survival. Annual adult survival is currently being estimated in two of the 12 monitoring areas (Finnmarksvidda and Fauske, Figure 1) and is based on genetic monitoring. Adult individuals are given 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 annual adult survival. For further information about the general principles of using genetic analyses to estimate annual adult survival in raptors, see e.g. Rudnick *et al*. 2005.

Samples for DNA are collected in June and July as part of the summer field work. Moulted feathers are collected from and underneath nest and roosting sites, and blood sampled or developing feathers plucked from nestlings. Moulted feathers are stored in paper envelopes, the tip of nestling feathers are stored in 96% ethanol and blood samples are stored in lysis buffer at ambient temperature until being analysed. Genomic DNA are 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 (cf., 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 (Supplementary Table S1). Based on this set of markers, the probability of two individuals having the same DNA-profile by chance is low ($P_{ID} = 2.8\times10^{-12}$). 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). 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 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

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identification. Unique genotypes are identified by using the program allelematch (Galpern et al. 2012). Capture-mark-recapture methods are used to estimate annual adult survival.

DISCUSSION

Unbiased data on reproductive rates and survival allow comparisons among 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 like weather and prey supply (Steenhof & Newton 2007).

The methodology used to quantify reproduction for Golden Eagle in Norway deviate slightly from the methodology used in some other countries (e.g., Hardey *et al*. 2013, Steenhof & Newton 2007) but can still be used for comparison if used with caution and with an awareness of the diverging criteria. Most studies/monitoring programs of Golden eagle estimate breeding success as number of fledglings per occupied territories (e.g., Hardey et al. 2013, Steenhof & Newton 2007). However, for Norway we find it difficult, or near impossible, to determine if a territory is occupied or not. It would require an enormous field effort, often not possible due to economical limitations. This is especially difficult in non-breeding years. The criterion "flight display in the nesting area" 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 neighbour's territory (Walker 2017). Our data for the last two years of intensive monitoring show that about 8-10 % of the territories was classified as not occupied according to the criteria given above. However, as discussed, this might be an overestimate. Because of these uncertainties in the determination of occupancy in a territory, all territories and not only those documented as 'occupied territories', is used in the calculation of breeding success in Norway, i.e. we divide the number of chicks ≥ 50 days on the 15 preselected territories. Our estimate of breeding success allows for comparison of yearly variation in number of fledglings caused by fluctuating environmental conditions are capable of detecting both very good (breeding in all territories) and very bad years (few territories with breeding). If territories that do not meet the criteria of being occupied are excluded, the breeding success could easily be overestimated in bad years.

We regard chicks ≥ 50 days as the minimum requirement for a presumption of fledging in the Golden Eagle. This choice was made to allow for ringing of chicks in the nest while they were still small enough for handling. In Britain and North America, they use at least eight weeks (56 days) as a minimum age (Hardey *et al*. 2013, Steenhof & Kochert 1982). Our experiences suggest a very low mortality of chicks > 50 days while they are still in the nest. However, the exact age of chicks to determine successful breeding, 50 or e.g. 56 days is not that important as long as the same criteria is

used (here >50 days) when data are compared between areas and years. Thus some caution should be used when comparing breeding success data between countries where different criteria has 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 provide DNA with sufficient quality and quantity for genetic analysis to identify individuals (e.g., 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 (unpublished 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 constitute a powerful, as well as non-invasive, monitoring method that can be applied to obtain estimates on e.g. annual adult survival.

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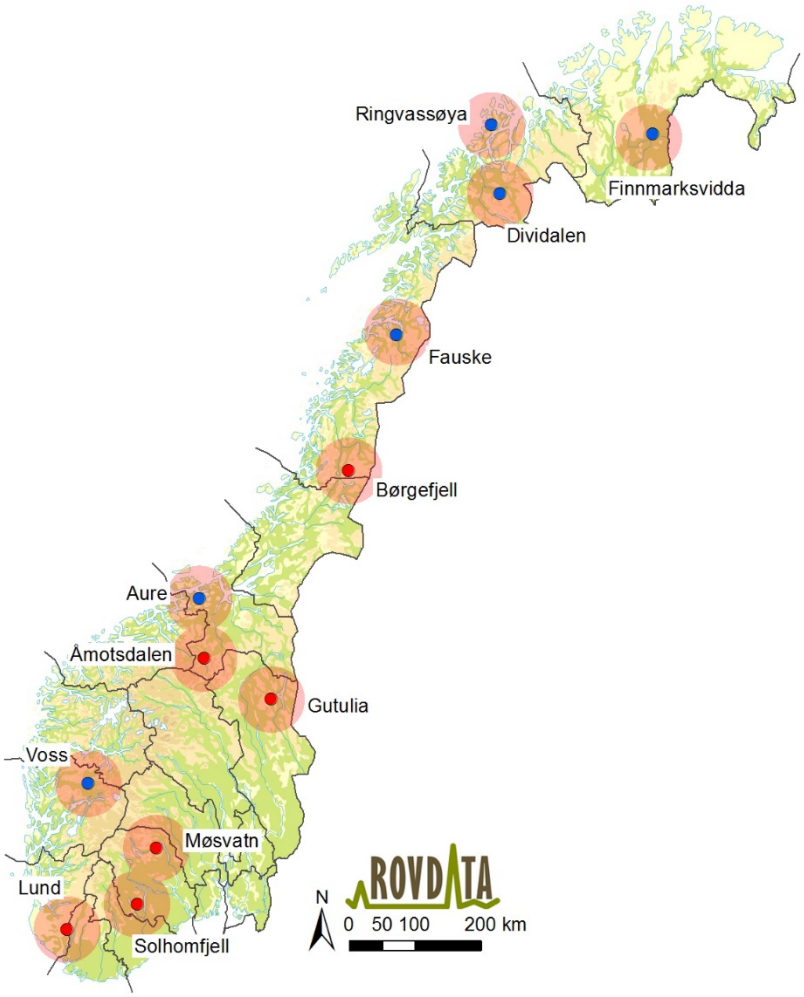

Figure 1

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Figure 2

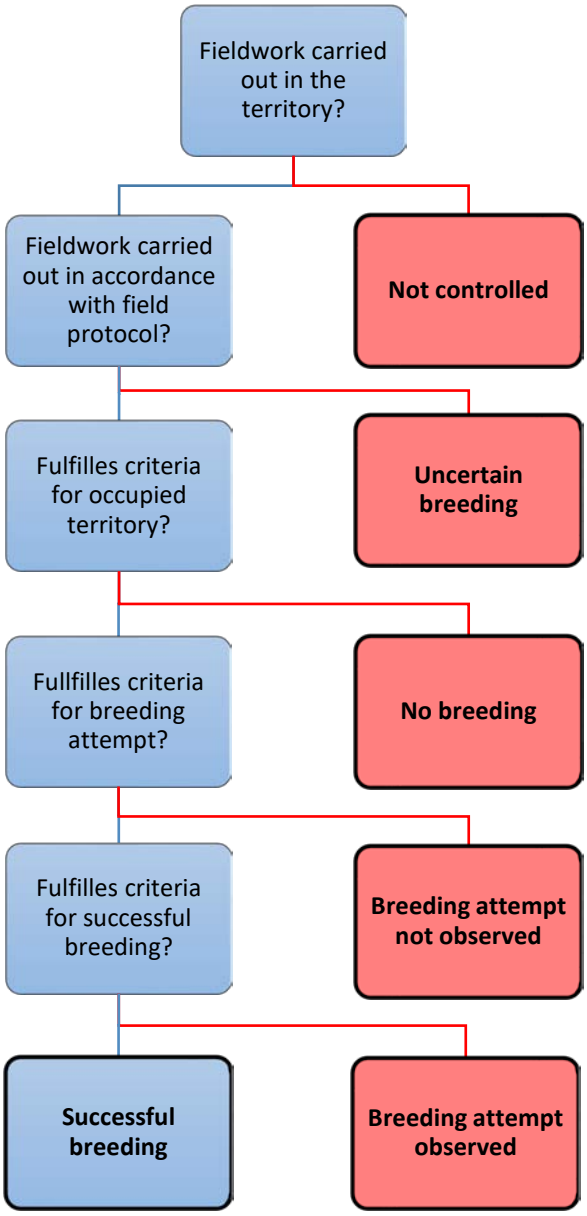

Figure 3.

Figure captions

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

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

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

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Online Supplementary Material

Table S1. Characterization of microsatellite loci used for individual identification of Golden Eagles.

Locus	Reference	MP set	n	A	H_O	H_E	P_{ID}
Aa02	Martinez-Cruz et al. (2002)	A	65	6	0.66	0.60	0.22
Aa04	Martinez-Cruz et al. (2002)	A	65	9	0.54	0.65	0.18
Aa15	Martinez-Cruz et al. (2002)	A	65	5	0.46	0.44	0.34
Aa26	Martinez-Cruz et al. (2002)	A	65	6	0.71	0.67	0.15
Aa39	Martinez-Cruz et al. (2002)	A	65	9	0.80	0.76	0.08
Aa43	Martinez-Cruz et al. (2002)	A	65	6	0.77	0.75	0.10
IEAAAG04	Busch et al. (2005)	A	65	8	0.88	0.79	0.07
IEAAAG15	Busch et al. (2005)	A	65	3	0.45	0.53	0.32
Aa12	Martinez-Cruz et al. (2002)	B	65	5	0.65	0.56	0.22
Aa27	Martinez-Cruz et al. (2002)	B	65	4	0.55	0.60	0.21
Aa36	Martinez-Cruz et al. (2002)	B	13	8	0.92	0.85	0.04
Bbu42	Johnson et al. (2005)	B	65	10	0.89	0.84	0.05
SNMS32	Hirai and Yamazaki (2010)	B	65	9	0.72	0.76	0.09
Z37B	Dawson et al. (2015)	B	37F	2	1	1	
			28M	1	0	0	

MP, multiplex set; n, number of genotyped individuals; A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; P_{ID}, probability of identity. F=females; M=males.

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