

Summary of PhD theses

Population viability analysis and dispersal characteristics of the eastern imperial eagle (*Aquila heliaca*) in the Carpathian Basin

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Budapest, 2025

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INTRODUCTION

Status and biology of the imperial eagle

The eastern imperial eagle (*Aquila heliaca*, SAVIGNY, 1823; hereafter referred to as 'imperial eagle') is a large-sized, territorial, long-lived raptor species of the forest steppe zone of Eurasia. It is listed as Vulnerable by IUCN, with an estimated maximum of 10,000 mature individuals globally. It is threatened by numerous anthropogenic factors, including poisoning, habitat destruction, electrocution, and illegal hunting. As it is an apex predator, its conservation is crucial for maintaining ecosystem health.

Imperial eagles prefer forest steppes and lowland agricultural areas for nesting. They usually start breeding in their fourth calendar year. Immature birds are floaters, exploring areas often hundreds or thousands of kilometres from their natal site. Once settled, they show high nesting site fidelity. They are considered genetically monogamous. During the breeding, females do most of the incubation, while males hunt for food. Their diet mainly consists of small and medium-sized mammals and birds.

The imperial eagle's distribution spans from Eastern Austria to Russia's Trans Baikal region and from the Southern-Ural Mountains to Turkey. The large eastern populations are migrants, while the smaller, isolated western populations in Central Europe, the Balkans, Turkey and South Caucasus are mainly sedentary.

The Pannonian (Carpathian Basin) population on the western edge is the largest unified population (ca. 550 nesting pairs as of 2022) outside of Russia and Kazakhstan. It can be divided into a larger Eastern (East Hungary, East Slovakia, Serbia and Romania) and a smaller Western (West Hungary, West Slovakia, Austria and the Czech Republic) subpopulation, with most pairs residing in East Hungary (ca. 400 pairs).

Due to agricultural intensification and persecution, both subpopulations experienced a bottleneck in the previous century, leading to only a few pairs remaining in mountainous areas by 1980. Following the decrease in persecution activity, the population started to expand and its productivity has begun to increase. The Eastern subpopulation has been growing more rapidly since, suggesting differences in the demographic parameters of the two subpopulations.

Population viability analysis (PVA)

Due to its size and peripheral placement in the species' range, the Pannonian population holds great significance for the conservation of the imperial eagle. Therefore, the aim of the present thesis was to conduct a population viability analysis (PVA) to examine possible future population growth and investigate which demographic parameters have the highest impact on population growth rate and viability.

PVA uses simulation models that approximate real population dynamics by incorporating demographic parameters and existing knowledge on the species and its environment. These models account for both deterministic processes and stochastic variation, providing a more realistic assessment of population viability. VORTEX is the most often used software for PVA, owing to its highly customisable individual-based model.

The recovery of the Pannonian population has been well documented since 1980 in terms of the number of nesting pairs and productivity. However, knowledge on survival rates and dispersal characteristics, including natal dispersal distances and migration rates, was limited. Therefore, the first part of the dissertation focuses on estimating these missing parameters.

Survival estimates of breeding imperial eagles

In long-lived species, population growth is generally most influenced by survival rates; thus, the accurate estimation of age-, life-stage- or sex-specific survival rates is crucial. In raptors, sex differences in survival are generally small, with either males or females having lower survival rates, with reasons usually attributable to behavioural differences.

In Hungary, a surprisingly low survival (72.3%) was estimated for breeding females between 1997 and 2006. This was later suspected to reflect undetected poisoning activity during that period. Poisoning—both intentional (directly aimed at predators) or accidental (resulting from improper pest control practices)—was the leading known mortality cause between 2005 and 2019. Its highest rates occurred in 2011–2014, coinciding with a stagnation in population growth. Nonetheless, survival rates and their relationship to poisoning remained poorly understood prior to this thesis.

Natal dispersal characteristics

Natal dispersal is the movement of an individual from its place of birth to the site where it first attempts to breed. Since it directly influences the distribution patterns of individuals, it has major implications for population dynamics, genetic structure, and colonisation potential.

Sex and conspecific density are two of the most important factors shaping dispersal patterns. In birds, dispersal is generally female-biased, which has been linked to their typically monogamous mating system. The relationship between density and natal dispersal is less understood due to complex driving factors and inconsistent results even within taxonomic groups. In raptors, both conspecific avoidance and attraction, as well as no density dependence, have been observed.

Previous GPS telemetry studies have suggested high natal philopatry in the imperial eagle. However, there was only scarce data published on natal dispersal distances and the factors shaping natal dispersal and settlement choices have not been studied prior to this thesis.

Monitoring methods

To estimate survival or dispersal rates, birds need to be individually identified, and their fate monitored over an extended period of time. However, applying conventional markings (i.e. rings, wing-tags or GPS transmitters) to large-sized raptors after fledging is extremely challenging. Therefore, these techniques can only be used to mark chicks in adequate numbers.

DNA profiling of the shed feathers collected from the territories provides a non-invasive alternative for identifying and monitoring adult birds. This method requires a set of genetic markers (e.g. microsatellites) that provide sufficient allelic variation to reliably distinguish individuals.

Microsatellite markers (STRs) have previously been used to study population dynamics in the imperial eagle. However, the resolution of these marker sets was adequate only for individual identification in smaller populations and needed to be improved to enable effective individual monitoring in the Pannonian Region as well.

AIMS OF THE DISSERTATION

1. To **assemble a microsatellite marker set** that ensures the reliable DNA-based individual identification of imperial eagles in the Pannonian population, thereby supporting the study of demographic parameters.
2. To **estimate annual survival rates for breeding imperial eagles** in 2011–2022 in East Hungary, using a mark-recapture method based on DNA profiling. We aimed to explore possible sex differences in survival and investigate the relationship between poisoning rates and annual survival probabilities.
3. To study **natal dispersal behaviour** in the Pannonian population in 2011–2024 using colour-ringing, GPS tracking and DNA profiling methods. We aimed to study natal dispersal patterns in relation to sex, local density and dispersal direction.
4. To **conduct a population viability analysis (PVA)** for the Pannonian population, employing the previously estimated survival and dispersal rates and published reproductive parameters. We aimed to give predictions for future population growth in East Hungary, assess the possible differences in the demographic parameters of the Eastern and Western subpopulations, and investigate which parameters have the highest impact on population growth.

MATERIALS AND METHODS

This thesis is grounded in 40 years of monitoring coordinated by MME BirdLife Hungary. Annual number of nesting pairs and reproductive parameters have been thoroughly recorded since 1980. In addition to the conventional marking methods of ringing and GPS tracking, we also used genetic monitoring. This included DNA profiling chicks from plucked feathers and breeding individuals from their shed feathers collected at the nest sites, using a minimum of nine microsatellite loci. Sex was determined via molecular sexing.

Improving the microsatellite marker set

We tested 26 cross-species microsatellite markers: six from the Spanish imperial eagle (*A. adalberti*), 13 from the Japanese golden eagle (*A. chrysaetos japonica*) and seven from the white-tailed eagle (*Haliaeetus albicilla*).

Markers were first tested in monoplex PCR reactions on the DNA samples of 4–15 unrelated adult birds. Polymorphic markers with adequate amplification properties were then organised into multiplexes to reduce genotyping costs. We tested the markers for Hardy-Weinberg equilibrium and the presence of null alleles, and estimated the probability of identity (i.e. the resolution of the marker set).

Survival estimation of breeding birds

We conducted a mark-recapture analysis based on genetic monitoring to estimate the annual survival probabilities of 208 males and 411 females in East Hungary between 2011 and 2022. We identified adults mainly from their shed feathers, but in some cases, we also used the plucked feathers of their chicks to infer their presence via parentage analysis. We examined the effects of sex and poisoning rate on survival.

We used the Cormack-Jolly-Seber mark-recapture model in MARK within the RMark framework. Model selection was based on QAICc values (AIC corrected for small sample size and overdispersion).

Natal dispersal characteristics

Between 2011 and 2022 in Hungary, 1660 imperial eagle chicks were tagged with ornithological and colour-rings, 71 chicks were also equipped with GPS transmitters, and 631 were DNA profiled to allow for their identification from shed feathers once they settle to breed.

We studied natal dispersal patterns in regard to sex, local density (natal site or breeding site density), and dispersal direction. We defined local density at each nest site as the

reciprocal of the area of the Thiessen polygon drawn around the nest.

We used general linear mixed models to examine the relationships between natal dispersal distance, sex, natal site density and breeding site density. Rayleigh tests were used to observe patterns in dispersal direction.

Population viability analysis (PVA)

We constructed a stage-based PVA model in VORTEX, where each stage referred to a certain age and breeding status (floater or nesting). In each year of the simulation, individuals progressed between stages, produced offspring and dispersed between subpopulations based on previously estimated demographic parameters of survival, productivity and dispersal.

Values of the uncertain parameters of immature survival and dispersal rate were determined using sensitivity tests, during which those values were chosen that best reproduced the observed population trajectory of East Hungary between 1980 and 2022.

We conducted a similar test to determine the most likely values of mortality rates in the whole Eastern and Western subpopulations relative to the mortality in East Hungary.

We also investigated future population growth under different mortality and reproductivity scenarios for East Hungary for the period of 2022–2064.

Using elasticity analysis, we determined which demographic parameters (breeding failure, first-, second-, third-year or nesting mortality) have the greatest impact on population growth and should therefore be in the focus of conservation.

We ran each simulation for 250 iterations. We used the means and standard deviations of the demographic variables (number of individuals, number of nesting pairs, number of offspring) to compare models with different parameter settings (scenarios).

RESULTS

Improving the microsatellite marker set

We selected 17 markers for the final marker set, seven previously used and ten newly tested markers (two imperial eagle, ten Spanish imperial eagle, three Japanese golden eagle and two white-tailed eagle markers). All markers were in Hardy-Weinberg equilibrium, and null alleles were not detected. The new marker set provides eight orders of magnitude higher resolution than the former set (the probability of identity was reduced from 10^{-6} to 10^{-14}).

Survival estimation of breeding birds

Several models received similar support during model selection ($\Delta\text{QAICc} < 2$).

The minimum QAICc model estimated survival as a constant probability over time with a $91.6\% \pm 0.8\%$ SE annual survival independent of sex or poisoning rate.

Estimates of the second most-supported model showed a negative relationship between survival and poisoning, with a $92.9\% \pm 1.3\%$ SE survival for the interval with the lowest poisoning rate (0.46%, 2017–2018) and a $88.4\% \pm 3.1\%$ SE survival probability for the interval with the highest poisoning (4.68%, 2011–2012).

In the model where survival was assumed to be sex-dependent but constant in time, males were estimated to have a lower survival probability than females. However, this difference was small (males: $90.9\% \pm 1.8\%$ SE, females: $91.8\% \pm 0.9\%$ SE), and the CI for the coefficient of the sex effect overlapped zero.

The model with an interaction between sex and poisoning rate received somewhat more support. Based on its estimates, only male survival was affected significantly by poisoning ($\beta = -0.507$, 95%CI = -0.927 to -0.087).

Natal dispersal characteristics

We detected high natal philopatry, with no emigration from the Pannonian population and only 4.3% (five individuals) dispersing from the Eastern to the Western subpopulation.

Natal dispersal distances were longer for females (median 57.6 km, 95% CI 46.4—69.7 km, $n=72$) than for males (median 35.9 km, 95% CI 22.9—45.6 km, $n=43$).

We found a negative relationship between density difference (i.e. the difference between breeding site and natal site density) and natal site density ($p<0.0001$). In general, birds dispersed to lower density breeding sites compared to their natal site, but dispersed to higher density breeding sites when their natal site density was low.

Rayleigh tests revealed that females hatched in the second period of the study (2015–2020) preferred to disperse in a southern direction ($p = 0.004$, $n = 40$).

Population viability analysis (PVA)

Using our estimates of demographic parameters, the model successfully reproduced the population growth observed in East Hungary since 1980. The results indicate that the Pannonian population is self-sustaining and likely recovered without substantial immigration.

Based on sensitivity tests, the best fit to the observed trajectories of the subpopulations was achieved when mortality was set 5.5% higher for the Eastern subpopulation and 35% higher for the Western subpopulation relative to the East Hungarian mortality values.

The probability of extinction until 2064 was zero for all examined future scenarios. Under current mortality rates and average productivity, the East Hungarian part of the population is expected to reach the estimated carrying capacity of 1310 nesting pairs by 2038. If mortality rates increased by 60%— as during peak poisoning compared to years with minimal poisoning —the population would grow more slowly and stabilise below the carrying capacity, with all the three investigated productivity levels. Population stagnation would occur if mortalities across all stages rose by 90%, 83%, and 70% under high, average, and low productivity, respectively.

Elasticity analysis showed that breeding failure, mortality of nesting birds and first-year mortality have the highest impact on the population growth rate.

CONCLUSIONS

The Pannonian population holds particular importance for the conservation of the imperial eagle, owing to its positive population trend and peripheral placement in the species' range. Thanks to the continuous and extensive monitoring over the last four decades, we were able to address knowledge gaps on survival rates, natal dispersal behaviour and population dynamics.

Adult survival is a highly influential demographic parameter in raptors, as also suggested by our elasticity analysis. We estimated >90% mean annual survival for breeding birds in East Hungary in 2011–2022, which value is typical of a healthy population of large raptors.

We found some evidence for the negative relationship between poisoning rates and adult survival. Mortality rates were estimated to have risen by ~60% at peak poisoning activity compared to years with minimal poisoning—an increase that, if affecting all age classes, could significantly reduce the population growth rate in the future, as indicated by our PVA projections. Poisoning also seemed to have a higher impact on male survival, likely explained by their increased foraging activity compared to females during the chick-rearing period, when most poisoning incidents occur.

Natal dispersal behaviour also plays a key role in population dynamics. Our findings confirmed the expected female-biased natal dispersal, consistent with both theory and empirical evidence from birds. This sex bias could function as a mechanism of inbreeding avoidance, potentially contributing to the maintenance of the previously reported high genetic diversity despite the former bottleneck. The males' shorter dispersal underlines their importance in colonisation dynamics, especially if we consider their potential vulnerability to poisoning.

The correlation between natal and breeding site densities is rarely examined, but here we showed that although imperial eagles generally disperse toward lower density areas compared to their natal site (as a means of competition avoidance), they are also attracted to the presence of conspecifics.

Conservation relevance

The detected high philopatry and PVA-based evidence of self-sustainability together suggest that the Pannonian population is largely isolated. Such isolation increases the risk of inbreeding and, consequently, inbreeding depression.

However, considering the continued expansion of the Pannonian population, its isolation and the associated risk of inbreeding is expected to lessen over time. The southward

expansion towards Serbia promotes connectivity with the nearest populations in Bulgaria and North Macedonia. Serbia contains extensive potential habitat for imperial eagles, as the population still has not recovered following its extirpation in the last century. Strengthening habitat protection and species conservation in Serbia is therefore crucial for supporting population recovery and interconnectedness. In addition, the establishment of stepping-stone populations in these areas may also accelerate the population's expansion and enhance long-term connectivity.

When connecting these populations, a potential concern is that their genetic isolation may have led to local adaptations, which could cause individuals of mixed origin to have reduced fitness, a phenomenon called outbreeding depression. However, outbreeding depression is generally not a concern in same-species populations from similar environment. Additionally, the imperial eagle's high flexibility in prey selection suggests a strong capacity to adapt to environmental changes.

While the Pannonian population is highly viable at the current demographic parameters, population growth may be hindered by elevated levels of poisoning activity. Thus, we advocate for the continued practice of the successful anti-poisoning measures implemented by Helicon LIFE and

PannonEagle LIFE projects in Hungary and in the neighbouring countries as well. In addition, monitoring should focus on any possible source-sink dynamic between the two subpopulations, as highlighted by the potentially unbalanced dispersal rates and the possible mortality differences suggested by the PVA. For such studies, our microsatellite marker set could prove valuable.

NEW SCIENTIFIC RESULTS

Methodological results

1. **Microsatellite markers** of the white-tailed eagle (*Haliaeetus albicilla*) and the Japanese golden eagle (*Aquila chrysaetos japonica*) can be utilised for the individual identification of the imperial eagle.
2. We assembled the **highest resolution microsatellite marker set** currently available for the imperial eagle, enabling more reliable individual identification and relatedness estimation in the future.

Ecological results

3. Using a mark-recapture method based on genetic identification from shed feathers, we managed to estimate the **annual survival probabilities** of both male and female breeding birds in East Hungary for 2011–2022.
4. We found moderate evidence that **poisoning activity may lead to a male-biased mortality** in breeding birds, probably attributable to the behavioural differences of the sexes.
5. We report that **natal dispersal distances** in the imperial eagle are female-biased.
6. Our study on the **density dependence of natal dispersal** suggests that both competition avoidance and

conspecific attraction influence settlement decisions in the imperial eagle.

Modelling results

7. We constructed a population model which, using our estimates of demographic parameters, successfully reproduced the population growth observed in the Pannonian Region since 1980. This implies that the **Pannonian population is self-sustaining**, as its recovery following the bottleneck in the 20th century was possible without significant immigration from other distant populations.
8. Results of the PVA suggest that **mortality rates** in the Western subpopulation and East Slovakia may be higher than in East Hungary.
9. Based on elasticity analyses, proportional changes in breeding failure, nesting mortality and first-year mortality have the **highest impact on population growth rates**.
10. The population in East Hungary increases by 11.5% each year and is expected to **reach the carrying capacity by 2038**. Increase in mortality rates due to elevated poisoning activity would hinder population growth rates, but with the current values of reproductive parameters, the population is not expected to decline up to an 83% increase in mortality rates across all age groups.

PUBLICATIONS RELATED TO THE DISSERTATION

1. Full-text publications in peer-reviewed journals with an impact factor

Zsinka B, Vili N, Szabó K, Tisza Á, Csonka V, Pásztory-Kovács S (2024) Mikroszatellita-markerkészlet fejlesztése parlagi sasok (*Aquila heliaca*) egyedi azonosításához rokon fajokban leírt markerek segítségével. *Magy Állatorvosok Lapja* 146:357–365.

Zsinka B, Pásztory-Kovács S, Kövér S, Vili N, Horváth M (2024) Moderate evidence for the sex-dependent effect of poisoning on adult survival in a long-lived raptor species.

Zsinka B, Kövér S, Horváth M, Vili N, Szabó-Csonka V, Szabó K, Pásztory-Kovács S (2025) Sex-Biased and Density-Dependent Natal Dispersal in a Highly Mobile but Philopatric Raptor. *Ecol Evol* 15:e71487.

2. Oral presentations at international and Hungarian conferences

Csonka V, **Zsinka B**, Horváth M, Vili N (2021) The effect of sex and local density on the natal dispersal of eastern imperial eagles in the Carpathian Basin. Magyar Etológiai Társaság XXIII. Konferenciája, Budapest, Hungary, 26-27 November 2021.

Zsinka B, Kövér S, Csonka V, Vili N, Szabó K, Fatér I, Juhász T, Horváth M, Pásztory-Kovács S (2023) Genetic monitoring of Imperial Eagles in the Pannon region. PannonEagle LIFE Closing Conference, Jászberény, Hungary, 27 January 2023.

Zsinka B, Horváth M, Pásztory-Kovács S, Kövér S (2024) A parlagi sas (*Aquila heliaca*) populációéletképességi elemzése a Pannon régióban. In Lőrinczi G, Tölgyesi C (eds.): 13. Magyar Ökológus Kongresszus – Előadások és poszterek összefoglalói (p. 88.). Szeged, Hungary, 21-23 August 2024.

Zsinka B, Csonka V, Tisza Á, Szabó K, Vili N, Pásztory-Kovács S (2024) Parlagi sasok (*Aquila heliaca*) egyedi azonosítására használt mikroszatellita markerkészlet továbbfejlesztése. In MTA Állatorvos-tudományi Bizottsága Akadémiai Beszámolók: Állathigiénia, Állattenyésztés, Genetika, Takarmányozástan (p. 7.). Budapest, Hungary, 29-31 January 2024.

Zsinka B, Kövér S, Pásztory-Kovács S, Horváth M (2024) Population viability analysis (PVA) of the eastern imperial eagle (*Aquila heliaca*) in the Pannonian Region. In Student Conference on Conservation Science 2024 Book of Talk Abstracts (p. 5). Cambridge, UK, 26-28 March 2024.

3. Poster presentations at international and Hungarian conferences

Zsinka B, Kövér S, Pásztor-Kovács S, Vili N, Szabó K, Fatér I, Horváth M (2022) Survival estimation of eastern imperial eagles (*Aquila heliaca*) in Hungary with a genetic-based mark-recapture method. In Zasadil P, Ludvíková V, Báldi A (eds.): Book of Abstracts – 6th European Congress of Conservation Biology (p. 141). Prague, Czech Republic, 22 – 26 August 2022.

Zsinka B, Horváth M, Pásztor-Kovács S, Kövér S (2025) Population Viability Analysis of the Eastern Imperial Eagle (*Aquila heliaca*) in the Pannonian Region. In: Zsinka B, Süle G, Báldi A, Palotás B (eds.) 10th Student Conference on Conservation Science, SCCS Europe - Connecting Eastern and Western Europe in conservation biology, Abstracts. Balatonvilágos, Hungary, 2-6 September, 2025.

PUBLICATIONS NOT RELATED TO THE TOPIC OF THE THESIS

1. Full-text publications in peer-reviewed journals with an impact factor

Kristensen AU, Zsinka B, Lang Z, Hetényi N (2023) Survey of the Husbandry, Health, and Welfare of Norwegian Pet Rabbits. *J. Adv. Vet. Res.* 13:767-775.

Gilián LD, Endrédi A, Zsinka B, Neményi A, Nagy JG (2019) Morphological and reproductive trait-variability of a food deceptive orchid, *Cephalanthera rubra* along different altitudes. *Appl. Ecol. Environ. Res.* 17:5619-5639.

2. Full-text publications in peer-reviewed journals without an impact factor

Nagy JG, Zsinka B, Verebélyi V, Zorkóczy OK, Tyler T (2017) A *Vaccinium microcarpum* (Turcz. ex Rupr.) Schmalh. Magyarországon. *Kitaibelia* 22: 71-76.

3. Other publications

Zsinka B., Süle G, Báldi A, Palotás B (eds.) (2025): 10th Student Conference on Conservation Science, SCCS Europe - Connecting Eastern and Western Europe in conservation biology, Abstracts. Balatonvilágos, Hungary, 02 – 06 September 2025.

Zsinka B., Báldi A, Vajna F, Balogh E, Palotás B, Mázsa K (eds.) (2023): 8th Student Conference on Conservation Science, Balatonvilágos 2023: SCCS Europe - Connecting Eastern and Western Europe in conservation biology Abstracts. Balatonvilágos, Hungary, 13 – 16 September 2023.