Genetic monitoring and survival probability estimation of the Eastern Imperial Eagle in the Pannonian Region

Research Report for „Conservation of the eastern imperial eagle by decreasing human-caused mortality in the Pannonian Region (LIFE15/NAT/HU/000902)" project

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UNIVERSITY OF VETERINARY MEDICINE BUDAPEST, DEPARTMENT OF ECOLOGY

MOLECULAR ECOLOGY RESEARCH GROUP, AQUILABOR

Budapest 1077, Rottenbiller u 50. tel: 478-4213/8753 e-mail: ate.aquilabor@gmail.com

Report complied by:

Bernadett Zsinka, Szilvia Pásztory-Kovács and Szilvia Kövér

University of Veterinary Medicine Budapest, Department of Ecology, Molecular Ecology Research Group

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I.

Genetic monitoring of the Eastern Imperial Eagle in the Pannonian Region

Methods

Sample collection

We analysed shed feathers from approximately 200 breeding territories from the Carpathian Basin in each year between 2017 and 2022. Altogether approximately 5000 feathers were collected from the 400 Hungarian territories, furthermore we received samples from Slovakia, the Czech Republic, Austria and Serbia.

DNA-samples for genetic-profiling were collected in each year during the annual chick ringing and nest monitoring between June and September. Armpit feathers were plucked from nestlings during ringing, while breeding individuals were non-invasively sampled by collecting their shed feathers around the 100 m radius of the nest. This collection method has previously been proved to be reliable in sampling the resident birds, as in an earlier study, only 2% of the feathers collected this way were found to be originating from intruders and not from the breeding pair (Jakab, 2017). Plucked feathers were stored on -20°C in 2ml microtubes filled with 96% ethanol, while shed feathers were stored in tagged plastic bags in dark, dry, cool places in order to preserve DNA (Vili et al., 2013) and were processed as soon as possible, preferably in the year of collecting.

DNA extraction

We extracted the full genome DNA from 2242 feathers using the Omega E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek Inc.) following the manufacturer's instructions but using an additional 20 µl of dithiotreitol (1M) during the digestion step (Weigman, 1968). DNA was extracted from the tip of the calamus in plucked nestling feathers and from the superior umbilicus region in moulted feathers (Horváth et al. 2005).

Molecular sexing

Each individual feather was sexed by amplifying introns of the sex chromosome-linked CHD1 gene, using the primers (F2250/R2787, i16F/i16R; Fridolfsson and Ellegren 1999, Suh et al., 2011). The PCR reaction included 0.065 µl DreamTaq polymerase (Fermentas), 1.7 µl 10X DreamTaq Green puffer (Fermentas), 0.65 µl 25 mM MgCl2 (Thermo Scientific), 0.65 µl 2 mM dNTP mix (Thermo Scientific), 1-1 µl 10 pmol/µl forward és reverse primer, 8 µl H2O and 4 µl ca. 50 ng/µl concentration DNA. The PCR program for molecular sexing constituted of an initial denaturation step on 95°C for 2 minutes, a touchdown section of 9 cycles (denaturation: 95°C for 30s, annellation: temperature lowering by 1°C each cycle from 60-52°C and lasting 45s, elongation: 72°C for 45s), followed by 28 cycles of 95°C for 30s, 52°C for 45s and 72°C for 45s, ending with a final elongation of 7 minutes on 72°C. The PCR products were visualized through gelelectrophoresis (2% agarose gel stained with EcoSafe (Pacific Image Electronics Co., Ltd) intercalator, 100 V, 45 minutes) by UV illumination: the heterogametic females display two bands while the homogametic males only one.

Individual genotyping

For individual identification we need to include as much variable microsatellite loci as possible, thereby we can reach the appropriate resolution and easily can distinguish even the closely related individuals (e.g.: parent-offspring or siblings). Therefore, we selected the most reliable nine loci among the previously tested available microsatellite loci to identify the breeding individuals and study the sampled population. We selected two tetranucleotide loci (IEAAAG09 / G09 and IEAAAG11 / G11, Busch et al. 2005) optimized for Eastern Imperial Eagle and seven dinucleotide loci: five (Aa02, Aa35, Aa36, Aa39 and Aa43, Martínez-Cruz et al. 2002) were published for Spanish Imperial Eagle (*Aquila adalberti*) and two (Hal04 and Hal10, Hailer et al. 2005) for White-tailed Eagle (*Haliaeetus albicilla*).

If we had matching genotypes in two different territories, we involved further markers (Aa49, Aa53, Aa56, Martínez-Cruz et al. 2002; AQJ84, Naito-Liederbach et al. 2021) to exclude genotyping errors and confirm the breeding dispersal attempt.

Forward primers were 5'-labeled with fluorescent dyes (FAM6 ™, NED ™ or HEX). The 5' end of the forward primers were modified with the following fluorescent dyes (Applied Biosystems™): 6-FAM ™ for Aa02, Aa39, Aa43, G09, G11 and AQJ84, HEX ™ for Aa35, Aa36

and Aa56 and NED™ for Hal04, Hal10, Aa49 and Aa53. The 5' end of the reverse primers were modified with a pigtail 5'GTTT sequence in the case of Aa02, Aa36, Aa39, Aa49, Aa53, Aa56, AQJ84. PCR reactions were performed in a 10 µl volume, containing 10-70 ng of template DNA, 2 µl 5xFIREPol® Master Mix (Solis BioDyne), which consists of dNTP-mix, $MgCl_2$ and Taq DNA-polymerase for singleplex reactions. Aa36 and Aa39, Aa35 and Aa43, Aa49 and Aa53, Hal04 and Hal10, IEAAAG09 and IEAAAG11 were amplifiable as multiplexes as well. For the Hal loci, we used the PCR profile described by Hailer et al. (2006), with some modifications (37 cycles, 45 seconds for both annealing and amplification). A modified version of the PCR procedure described by Martinez-Cruz et al. (2002) was used in all IEAAAG and Aa loci, with a touchdown annealing scheme (decreasing from 66 °C to 50°C by 1°C in each step). The PCR program for the AQJ84 locus described by Naito-Liederbach et al (2021) was also modified to contain a touchdown sequence (decreasing from 66 °C to 55°C by 1°C in each step). Capillary electrophoresis was used to determine the fragment lengths. PCR products were run on an ABI3130 sequencer (Applied Biosystems, using Gene Scan TM -500LIZ TM Size Standard), alleles were identified and scored with Peak Scanner Software v.1.0 (Applied Biosystems , Foster City, CA, USA) and OSIRIS (NCBI). Fragment analysis was performed similar to the suggestions of Beja-Pereira et al (2009) by scoring each sample three times independently. Genotypes were assigned blind to the origin of the sample. Possible occurrence of null alleles and allelic dropouts were checked using MICROCHECKER 2.2.2 (Van Oosterhout *et al.*, 2004). Basic statistics (e.g. H_o, H_e) and PI (Probability of Identity), PI_{SIB} (Probability of Identity among siblings) values were calculated with GENALEX v.6.503 (Peakall & Smous 2012). Parentage analysis was performed both manually and using CERVUS 3.0 (Kalinowski et al. 2007).

Results

The genetic monitoring objectives of the project were met by identifying over 175 breeding individuals in each year during the study period 2017-2022 (Table 1). If we had more than one feather samples from a territory, we extracted the DNA from more feathers and we performed the molecular sexing, until we had found both the male and the female member of the breeding pair. If in the given year, we did not have samples neither from the female, nor from the male, we used the samples of their chicks: after the parentage analysis we could identify the local breeders or make decision about the exchange of the breeding pair in the territory. The total number of individual breeders identified in a year (Table 1) are the sum of breeders identified from moulted feathers and from chick genotypes via parentage analysis. Additionally to the samples listed in Table 1, we further genotyped 500 chicks hatched between years 2011-2016 with the aim of studying dispersal rates, a key aspect of population viability analyses.

Microsatellite marker set for individual identification

For the overall dataset, altogether 70 alleles were found at the 9 + 4 loci. Number of alleles per locus ranged between three (AQJ84) and ten (Aa35), with an average of 5.3 (detailed see in Table 2). PI_{SIB} for the 9-marker set was estimated to be 5.4×10^{-4} and 7.3×10^{-5} for the 13-marker set. P1X and P2X calculated for the 9-marker set were 0.998 and 0.996, respectively. Deviations from Hardy-Weinberg equilibrium with the possible presence of nullalleles were detected for locus Aa36 and thus a hetero-/homozygote difference between the parent and the offspring genotypes was allowed for this locus during parentage analyses.

Table 2. Microsatellite markers used for individual identification and their allele numbers.

Microsatellite markers used for all samples									
No. of alleles	Aa02	Aa35	Aa36	Aa39	Aa43	Hal04	Hal10	IEAAAG09	IEAAAG11
	6	10	6	8	7	5	5	4	4
Additional markers used for confirming or disproving the matches between genotypes									
No. of alleles	Aa49	Aa53	Aa56	AQJ84					
	4	5	3	3					

Table 1. Sample sizes in different stages of genetic analysis (preparation of feather samples, DNA isolation, sex determination, individual genotyping), the number of identified breeders and the number of chicks identified for parentage analysis in different countries during the years of the study period (2017-2022). Numbers belonging to project objectives mean the originally planned sample sizes of the project from different countries.

		HU	SK	AT	CZ	SRB	All countries
	Project objectives (no of. breeders to identify) in each year	*100*	*50*	*10*	*10*	*5*	*175*
	Individual breeders identified*	**239**	**12**	**3**	**4**	**0**	**258**
	Individual chicks identified (for parentage analysis)	41	12	6	5	0	64
2017	Samples prepared	438	30	14	16	0	498
	Samples isolated	438	30	14	16	0	498
	Samples successfully sexed	308	26	13	10	0	357
	Samples successfully genotyped**	*237*	26	11	9	0	283
	Individual breeders identified*	**157**	**25**	**5**	**5**	**0**	**192**
	Individual chicks identified (for parentage analysis)	18	4	1	1	0	24
2018	Samples prepared	331	51	16	14	0	412
	Samples isolated	331	30	16	10	0	387
	Samples successfully sexed	250	30	11	7	0	298
	Samples successfully genotyped	160	30	6	7	0	203
	Individual breeders identified*	**145**	**21**	**11**	**6**	**0**	**183**
	Individual chicks identified (for parentage analysis)	0	12	5	1	0	18
2019	Samples prepared	381	38	20	11	0	450
	Samples isolated	381	38	20	11	0	450
	Samples successfully sexed	329	36	17	10	0	392
	Samples successfully genotyped	156	32	16	10	0	214
	Individual breeders identified*	**158**	**8**	**5**	**5**	**0**	**176**
	Individual chicks identified (for parentage analysis)	19	0	0	0	0	19
2020	Samples prepared	302	12	17	10	0	341
	Samples isolated	302	12	16	10	0	340
	Samples successfully sexed	236	10	15	7	0	268
	Samples successfully genotyped	160	8	5	5	0	178

		HU	SK	AT	CZ	SRB	All countries
	Project objective (no of. breeders to identify) total	*100*	*50*	*10*	*10*	*5*	*175*
	Individual breeders identified*	**149**	**18**	**10**	**6**	**3**	**186**
	Individual chicks identified (for parentage analysis)	0	0	0	0	0	0
2021	Samples prepared	300	33	18	7	6	364
	Samples isolated	172	33	18	7	6	236
	Samples successfully sexed	156	32	15	6	5	214
	Samples successfully genotyped	152	18	10	6	3	189
	Individual breeders identified*	**199**	**21**	**4**	**7**	**0**	**231**
	Individual chicks identified (for parentage analysis)	0	0	0	0	0	0
2022	Samples prepared	436	46	7	14		503
	Samples isolated	265	46	6	14		331
	Samples successfully sexed	242	38	6	7		293
	Samples successfully genotyped	199	22	6	7		234

		HU	SK	AT	CZ	SRB	All countries
	Project objective (no of. breeders to identify) total	*600*	*300*	*60*	*60*	*30*	*1050*
	Individual breeders identified*	**1047**	**105**	**38**	**33**	**3**	**1226**
	Individual chicks identified (for parentage analysis)	78	28	12	7	0	125
Total	Samples prepared	2188	210	92	72	6	2568
	Samples isolated	1889	189	90	68	6	2242
	Samples successfully sexed	1521	172	77	47	5	1822
	Samples successfully genotyped	1064	136	54	44	3	1301

*including identification via parentage analysis

**some unsuccessfully sexed samples were also genotyped

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II.

Survival probability estimation for the Eastern Imperial Eagle breeding population in the Pannonian Region between 2011-2022

Introduction

Repeated identification of breeding individuals from their moulted feathers can be interpreted as capture-recapture attempts and therefore presence data gained this way can be analysed in a mark-recapture (CMR) framework in order to estimate survival. Here we aimed to estimate annual survival probabilities for breeding eastern imperial eagles in the East-Hungarian subpopulation during the LIFE projects (HELICON: LIFE10NAT/HU/000019 and PannonEagle) between 2011 and 2022. We investigated if survival differed by years or sexes. Sufficient amount of data was only available for the East-Hungarian subpopulation, thus survival probabilities were only estimated for birds breeding in this region. Data of the intensively sampled years 2011-2022 was supplemented with the data of year 2010, in order to increase the number of those captured individuals, whose recapture data can be used to estimate the survival probabilities of imperial eagles during the first years of the study.

Methods

Constructing capture histories

Capture histories (yearly presence-absence data for each individual) were constructed from two types of presences: direct presences, when a breeding bird was sampled and genetically profiled directly from its shed feathers and indirect presences, when the presence of a breeding bird was assumed through parentage analysis. Indirect presences served as additional presence data for a breeding individual in cases when it could not be sampled and

/ or identified from its shed feathers in the given year (shed feathers were not collected from that individual in that year or they were not processed in the lab or DNA-quality was not sufficient for constructing the genetic profile from the sample). Indirect presences could only be gained for an individual if it was previously profiled at least once and both the profile of the other member of the pair and the profile of at least one chick was known for the given year.

Capture histories for the 646 breeding birds (215 males and 431 females) included in the analysis consisted of a total of 1810 presences, 453 for males and 1357 for females, out of which 116 and 70 were indirect presences, respectively (Figure 1).

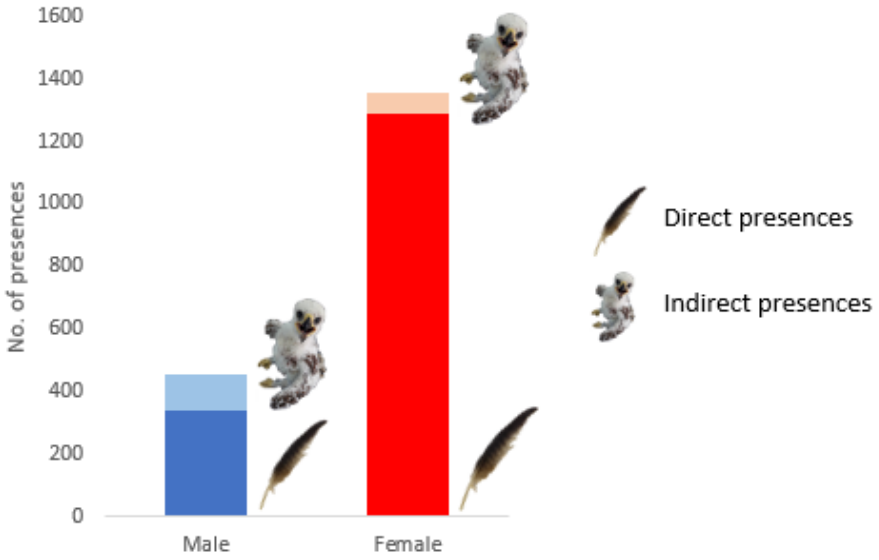

Figure 1. Distributions of direct and indirect presence data among males and females.

The sample sizes and the sampling efforts by sexes in each year are summarized in Table 1. Moulted feathers of the males can be found with a smaller probability around the nest, because of the different behaviour of the sexes during breeding, when only females incubate the eggs, while the males hunt and protect the territory. Males spending less time at the nest results in a smaller chance of finding their moulted feathers. Furthermore, sampling efforts were lower in later years relative to the population size, despite that the same amount or more birds got genotyped during these years, as the size of the population was increasing exponentially (Figure 2).

Table 1. Number of identified males and females, number of nesting pairs and relative sampling efforts (ratio of identified males or females and nesting pairs) in each year.

	Identified males	Identified females	No. of nesting pairs	Identified males / nesting pairs	Identified females / nesting pairs
2011	30	85	145	0.207	0.586
2012	34	62	151	0.225	0.411
2013	36	79	148	0.243	0.534
2014	32	92	152	0.211	0.605
2015	40	107	187	0.214	0.572
2016	34	121	205	0.166	0.590
2017	67	150	217	0.309	0.691
2018	34	113	250	0.136	0.452
2019	33	108	283	0.117	0.382
2020	42	113	329	0.128	0.343
2021	25	120	372	0.067	0.323
2022	38	156	386	0.098	0.404

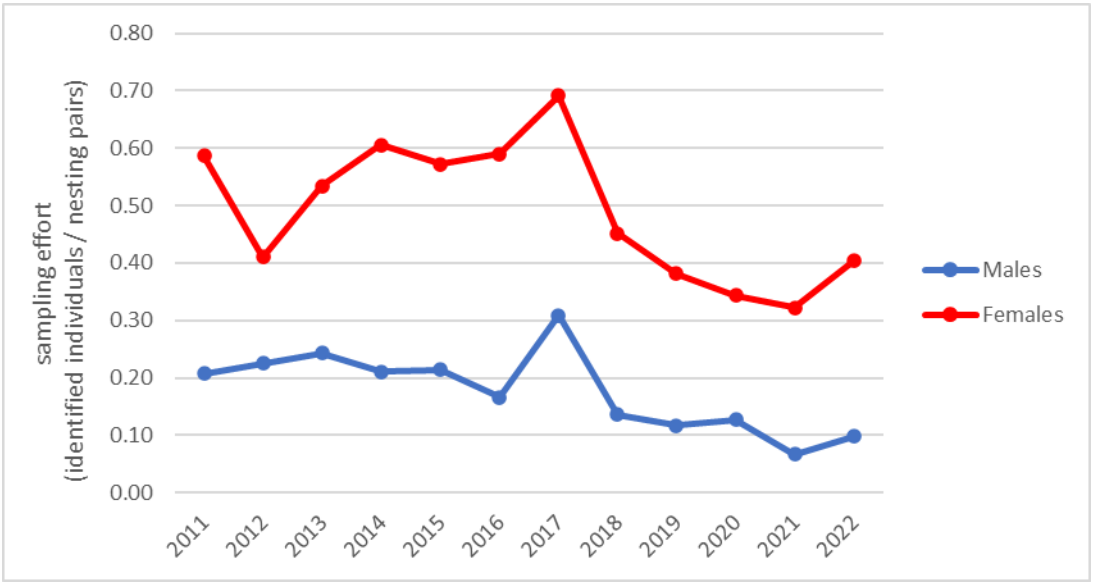

Figure 2. Relative sampling efforts (identified number of individuals / number of nesting pairs) for males and females in each year.

Survival analysis

Survival analysis was carried out in MARK v9.0 software (White & Burnham 1999) by fitting the Cormack-Jolly-Seber (CJS) open population model (Amstrup et al. 2010) to the capture histories. The mark-recapture method estimates annual survival probabilities (*Phi*) for each one-year interval and recapture probabilities (*p*) for each year as parameters of a generalized linear model. Sampling took place in June of each year, so survival probabilities refer to one-year intervals between June and June, e.g. interval 11-12 means: from June 2011 to June 2012.

The estimated survival is only an apparent survival probability, since the model cannot distinguish between actual mortality and emigration from the study population –, although since breeding imperial eagles have previously been found to display high nest site fidelity, this apparent survival can be considered as the actual survival probability. Survival estimates for the last time interval are highly unreliable by the nature of the method since this survival probability cannot be separated from the recapture probability of the last year.

The effects of sex and year on survival probability (*Phi*) and recapture probability (*p*) were examined by constructing models with different structures of sex and year-dependency (see Table 2 for candidate model set) and then choosing the best models through an AIC-based model selection procedure. In addition to models with fully year dependent survival (different estimate for each interval) and constant survival (same for each year), we also constructed models with simplified year-dependence (hereafter: year-sets) of survival by pooling the intervals into two sets (high and low survival sets) based on prior knowledge. The intervals within a set were constrained to have the same survival probability to decrease the number of parameters to be estimated. Both versions of year-dependence were combined with sex-dependence with or without an interaction term (sex * year, sex + year, sex * year-sets, sex + year-sets).

Since sampling effort differed between years and by sexes (Table 1, Figure 2), recapture probability (*p*) was always set to be sex- and year-dependent in the candidate model set with or without an interaction term (sex * year and sex + year), and also we built models with simplified year-dependence by pooling years with similar sampling efforts into sets, where years within a set were constrained to have the same recapture probability (sex * year-sets and sex + year-sets).

Assumptions of the Cormack-Jolly-Seber model include the homogeneity of survival and recapture probabilities among individuals and we used the goodness-of-fit tests of

program RELEASE 3.0 (Burnham, 1987) in order to inspect if these assumptions are met in our dataset. AICc values were corrected for the overdispersion parameter (c-hat) calculated from a bootstrap method (1000 simulations), resulting in QAICc values that were used in the model selection process. Models were considered significantly different if their delta QAICc values were more than 2 and the QAICc weights were used to evaluate the support for the competing models (Burnham & Anderson, 2002).

Table 2. Candidate model set of the mark-recapture analysis. Models were built from all the possible combinations of the listed survival and recapture probability settings. Years and intervals in brackets were constrained to the same estimate.

Survival probability (Phi)	
c	Constant over the years
sex	Different for males and females
year	Different between the years (different estimate for each year)
sex * year	Year-effect influences males and females differently.
sex + year	Year-effect influences males and females similarly.
year-sets [low survival] [high survival]	Different between two sets of intervals (different estimates for low and high survival intervals). Specific intervals are grouped together and constrained to the same estimate based on preliminary information (i.e. set of low survival intervals = intervals with high poisoning rates [11-12, 12-13, 18-19] and with low estimated survivals based on the fully year-dependent model [17-18]).
sex * year-sets [low survival] [high survival]	Different between two sets of intervals as above and also different by sex. Year-effect influences males and females differently.
sex + year-sets [low survival] [high survival]	Different between two sets of intervals as above and also different by sex. Year-effect influences males and females similarly.
Recapture probability (p)	
sex * year	Year-effect influences the sampling effort of males and females differently.
sex + year	Year-effect influences the sampling effort of males and females similarly.
sex * year-sets: [12] [11, 13, 14, 15, 16] [17] [18] [19, 20, 21, 22]	Year-effect influences the sampling effort of males and females differently along with recapture probabilities of specific years being constrained to the same estimate (based on previously calculated sampling efforts).
sex + year-sets: [12] [11, 13, 14, 15, 16] [17] [18] [19, 20, 21, 22]	Year-effect influences the sampling effort of males and females similarly along with recapture probabilities of specific years being constrained to the same estimate (based on previously calculated sampling efforts).

Results

Goodness-of-fit testing of models indicated a lack of fit of the Cormack-Jolly-Seber model to the data, with the overdispersion parameter c-hat estimated to be 5.468. The tests revealed the violation of the assumption of homogeneity of apparent survival and recapture probabilities, indicating that among birds that are identified in a given year, those birds that are identified for the first time are less likely to be seen later on than birds that have been identified previously. Adjusting for the overdispersion value results in increased uncertainty in parameter estimates and simpler models containing less parameters were favoured in model selection. The four best models are listed in Table 3. Model selection favored the models with recapture probability (*p*) being sex- and year-dependent without interaction and with specific years being constrained to the same estimates (model *p* (*sex + year-sets [12] [11, 13, 14, 15, 16] [17] [18] [19, 20, 21, 22]*). Estimated recapture probabilities of females were higher (0.408 – 0.789) than that of males (0.180 – 0.543) and were the highest in 2017 and the lowest in the last four years of the study (Figure 3). In the case of the best model, survival probability (*Phi*) was estimated to be constant, 0.917 [95%CI: 0.876; 0.946]. The second-best model included a year-dependency of survival, with the intervals between 2011-2012, 2012-2013, 2017-2018 and 2018-2019 having the same, lower survival estimate (0.891 [95% CI: 0.789, 0.948]) than the other intervals (0.931 [95% CI: 0.867, 0.965]) (model *Phi* (*year-sets [low survival] [high survival]*) (Figure 4). The third best model estimated survival to be sex-dependent, with males having a somewhat lower, 0.904 [95% CI: 0.795, 0.958] survival estimate than females: 0.921 [95% CI: 0.874, 0.951], although the estimate of males was highly uncertain and the confidence interval was fully overlapping with the conficende interval of the female estimate.

Table 3. Results of model selection including only the four best models, their Delta QAICc values compared to the best model, QAICc weights and the number of parameters.

Survival (*Phi*)	**Recapture probability (*p*)**	**Delta QAICc**	**QAICc weight**	**No. of parameters**
constant	sex + year-sets	0.000	0.488	7
year-sets [low survival] [high survival]	sex + year-sets	1.416	0.241	8
sex	sex + year-sets	1.863	0.192	8
constant	sex * year-sets	4.987	0.040	11

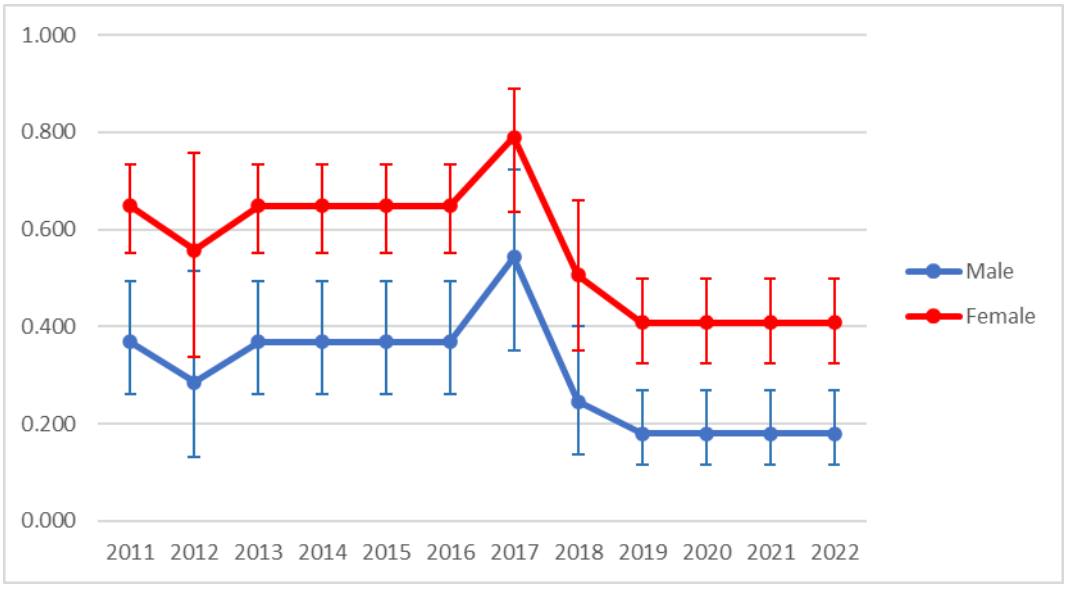

Figure 3. Recapture probabilities of males and females estimated from the best model, Phi (constant), p (sex + year-sets). Recapture probabilities of females were higher than that of males and were the highest in 2017 and the lowest in the last four years of the study.

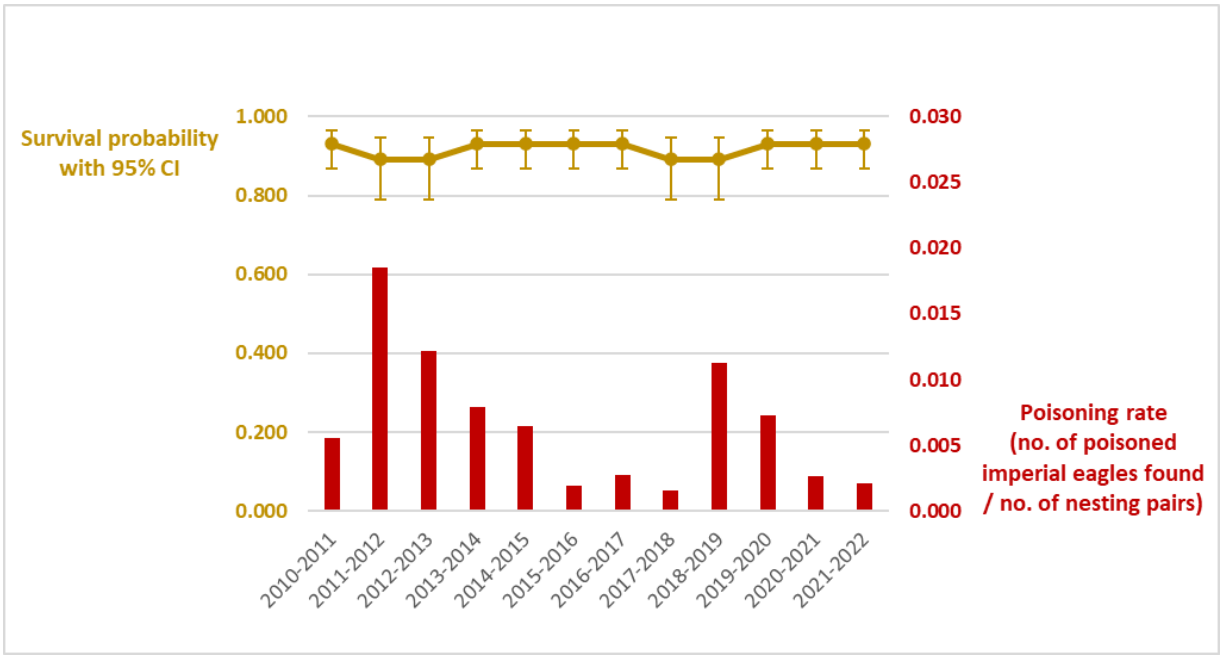

Figure 4. Survival probabilities of breeding birds estimated from the model Phi (year-sets [low survival] [high survival], p (sex + year-sets) along with poisoning rates (no. of poisoned imperial eagles found / no. of nesting pairs) for each interval. Survival estimates of 2011-2012, 2012-2013, 2017-2018 and 2018-2019 and that of the remaining intervals were restricted to the same esimates.

Discussion

The presented study aimed to estimate annual survival probabilities for breeding eastern imperial eagles in the Pannonian Region. Sufficient amount of information was only available from the East-Hungarian part of the population, therefore our results are applicable to birds breeding in this area.

Our best model estimated a 91.7% constant annual survival over the study period which is a reasonable survival rate for a large-sized raptor such as the imperial eagle (Newton et al., 2016.). Although this is only an apparent survival by definition – not distinguishing actual mortality and emigration -, we believe that since breeding imperial eagles exhibit high nest-site fidelity, apparent survival is very close to the real survival probability. We have no evidence for emigration from the Carpathian Basin population based on the GPS-tracking and the ringing data, furthermore models calculated from microsatellite data also did not support gene flow among European populations (Szabó et al., unpublished results).

Some support was found for differing survival probabilities between years. Regarding the year-dependent model, a four percentage point lower survival was estimated for the intervals 2011-2012, 2012-2013, 2017-2018 and 2018-2019 than for the remaining years. In the case of 2011-2012, 2012-2013 and 2018-2019 intervals this lower survival estimate conforms to the higher poisoning rates reported for these years. However, poisoning rate cannot provide an explaination for the lower survival of the interval 2017-2018, unless actual poisoning levels were much higher than that is reported. This is unlikely, since effort of detecting poisoning incidents was constant over the study period. Survival of breeding birds can depend on numerous, not independent factors other than human persecution, including electrocution, collision, prey availability, extreme weather such as heat waves, droughts, storms, unusually cold winter or spring days etc. Out of these factors prey availability and weather conditions can display high yearly variability that can contribute to yearly variability in survival.

It seems plausible that reported levels of poisoning resulted in a four percentage point reduction of survival, that can significantly affect population growth rate (see reports for Number of Nesting Pairs in Hungary 1980-2022, and for Population Viability Analysis). Reducing and eradicating this threat should still be in the focus of imperial eagle conservation programs. Even more so, assuming that poisoning might have a more drastic effect on the survival of the less experienced, younger age groups.

The model estimating sex-dependent survival has also gained some support, implying that survival of breeding males might be lower than that of females, however, the estimate for males was highly uncertain and the confidence intervals were entirely overlapping, thus we give little credibility for this result. Nonetheless, if male and female survivals are indeed different, it can lead to inequality in adult sex ratios, which can have a negative impact on population growth. A possible lower survival rate of males could be explained by the sexual dimorphism in behaviour during the breeding season: males spend more time hunting and protecting the territory and consequentially has more chance of coming in contact with human induced mortality factors such as electrocution or poisoning.

The same behavioural dimorphism explains the lower estimated recapture probabilities of males compared to females: they spend less time around the nest and so their moulted feathers are less likely to be found under the nest. Therefore, gaining additional indirect presences of breeding birds via parentage analysis turned out to be an especially useful tool in the case of males: about 25% of male presence data was generated this way.

The yearly sample size used for estimating survival has been relatively constant since 2015 (100-150 identified breeding birds in each year), yet this same sample size corresponds to a much lower sampling effort and recapture probability towards the end of the study due to the exponential growth of the population. Low relative sample sizes in the last years resulted in higher uncertainty of survival probability estimates for the last intervals in a fully year-dependent model (not shown here). In the future, after the PannonEagle Life program, with similar or even reduced resources we can only sample an even smaller proportion of the East-Hungarian population along with the increasing number of breeding pairs. Therefore we plan to keep the genetic monitoring only in some core areas, where the best tracked approx. 100 territories are located, as in these territories we can reach a high enough sampling effort for reliable survival estimates.

Goodness-of-fit testing of our Cormack-Jolly-Seber models revealed a heterogeneity in survival and recapture probabilities of birds identified at the same time, with the pattern of birds that are identified for the first time in the given year are less likely to be seen again later on than birds that have been identified previously. The dataset was also tested excluding indirect presences, since the method of how these presences are generated could introduce heterogeneity in recapture probabilities (indirect presences are only obtainable for birds that have been known from either a former or latter year). However, the above goodness-of-fit

issue was also apparent for the dataset that only contained presences gained from moulted feathers. We hypothesize that this pattern of heterogeneity might be due to an age-dependence in apparent survival that we could not explore in the recent study. Assuming that birds that are identified for the first time in a given year are new breeders and new breeders are mostly from the younger age groups, the reason why new breeders are less likely to be seen later on than older breeders could be explained by the lower apparent survival of younger birds. This could include a lower actual survival for the age group and a lower recapture probability due to the fact that they are prone to disperse to another breeding site after unsuccessful breeding attempts.

In conclusion, we estimated the annual survival of breeding imperial eagles in East-Hungary to be 91.7% on average. While the assumption of constant survival over the study period was the best-supported by model selection, some support for lower survival probabilities, mainly in years of high poisoning, was also found. Sex-dependent survival also received some support, the difference between male and female survival was approximately two percentage point, but the estimate of males was highly uncertain. The lack of fit of our models to the data implies that younger breeders might have lower apparent survival probabilities than do older breeders and thus we intent to include age-dependence in further analyses in order to estimate survival more reliably. Nonetheless, if the high poisoning rates reported between 2011-2022 indeed reduced the survival of breeders by four percentage point compared to years with less or no poisoning, then we emphasize that the further reduction of poisoning should be kept in the focus of future conservation of imperial eagles, as this magnitude of decrease in survival is known to have a significantly negative effect on future population growth of long-lived species.

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