# VARIATION IN OFFSPRING SEX RATIO OF A SEXUALLY DIMORPHIC, LONG-LIVED RAPTOR, THE EASTERN IMPERIAL EAGLE AT A PROTECTED NATURE RESERVE IN KAZAKHSTAN

## ВАРИАЦИИ В СООТНОШЕНИИ ПОЛОВ У ПТЕНЦОВ ОРЛА-МОГИЛЬНИКА В ПРИРОДНОМ ЗАПОВЕДНИКЕ КАЗАХСТАНА

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**Abstract.**

Management of protected areas impacts natural processes. Sex ratio theory attempts to explain observed variation in offspring sex ratio at both the population and brood levels. To assess the extent of skew in offspring sex ratios we evaluated the offspring sex ratio of 219 chicks in 119 broods at 30 territories of Eastern Imperial Eagles across seven years and four regions at a protected nature reserve in Kazakhstan. Only in one region in one year of our study did offspring sex ratio differ from parity (10 males: 1 female at 11 territories). Our results provide limited evidence of, and no mechanistic insight into, predictions associated with brood sex manipulation by these breeding populations of Eastern Imperial Eagles. However, they do suggest that protected areas should be large enough to accommodate fluctuations in demographic parameters such as brood sex ratio that have impacts at relatively small scales.

**Introduction.**

Protected areas are important to conservation of natural resources. Nevertheless, the ways in which protected areas protect or impact natural processes is not always clear. Sex ratio theory attempts to explain observed variation in offspring sex ratio, often the proportion of male offspring produced, both at the population and brood or litter levels. In the context of low-fecundity, high-investment organisms however, this relationship is often nonlinear (Frank 1990). In addition, natural selection predicts active primary (pre-laying or pre-birth) and secondary (post-laying or post-birth) brood or litter adjustments when possible. Both types of mechanisms are thought to occur in a complex response to environmental, individual, and social conditions (Trivers and Willard 1973, Charnov 1982, Bednarz& Hayden 1991, Wiebe&Bortolotti 1992, Hardy 2002, Bowers et al. 2013, Wojczulanis-Jakubas et al 2013) and both can interact with sibling competition resulting in siblicide to influence offspring sex ratios.

To assess the extent of skew in offspring sex ratios and to evaluate possible correlates of those skews to a long-lived avian species, we evaluated the sex ratio of offspring of Eastern Imperial Eagles (*Aquila heliaca*) over seven years at the Naurzum National Nature Reserve in north-central Kazakhstan. The Reserve is partitioned into separate ecological regions in which eagles show demographic and behavioural variability (Katzner, 2003, Katzner et al. 2005). Preliminary work has shown some landscape-wide variability in sex ratios across these ecological regions (Rudnick et al. 2005). In such cases, we expected bias towards lower cost males in response to variation in breeding region. Variation at this scale is an argument for increasing park size, to accommodate within-park variation in demographic parameters such as brood sex ratio.

**Methods.**

We conducted research at the NaurzumZapovednik (Naurzum National Nature Reserve) in the Kostanay Oblast of north-central Kazakhstan (51°N, 64°E). We use Zapovednik from here on to refer to the reserve as a whole. There are three distinct woodland patches–Tersec (T), Sip-sin (S),

and Naurzum–that encompass a majority of the reserve land. Because of ecological differences within the Naurzum forest, we further divide this woodland into two separate biotic regions–North and South Naurzum (NN and SN, respectively) (Fig 1; from Katzner et al. 2005). Specific differences among regions relate to distribution of prey species, to differences in eagle dietary patterns, and to differences in eagle nesting density (Katzner et al. 2005, Katzner et al. 2006a), and include previously reported regional differences in habitat use by eagles (Katzner et al. 2003). The remainder of the Zapovednik comprises dry steppe interspersed with predominantly ephemeral saline and fresh water lakes (Katzner et al. 2005).

The Eastern Imperial Eagle is a large, monogamous raptor with a geographic range that extends from eastern Europe into Siberia (Rudnick et al. 2005). In northern Kazakhstan the species is migratory and initiates the on-territory component of its breeding cycle in March (Katzner 2003). Nests are built in pine, birch, and occasionally aspen trees; conspecific nearest-neighbour distances among active nests average 2.1 – 2.7 km (SN and T) and 3.5 – 4.6 km (NN and S; Katzner et al. 2003). When breeding is successful, Eastern Imperial Eagles at the Zapovednik produce 1 – 3 chicks that fledge in early to mid-August, 71-80 days after hatching (Katzner 2003).

We surveyed established Eastern Imperial Eagle territories and new breeding sites over a seven-year period in spring 1998-2004. In July or August of each year we climbed to nests and plucked developing blood feathers from chicks that were close to fledging from the nest (generally ~50-65 days). Feathers were immediately placed in lysis buffer (100mM Tris-HCL ph 8.0, 100mM EDTA, 10mM NaCl, 2%SDS), and stored at room temperature for several months prior to -80°C storage (Rudnick et al. 2005). Genetic material in these plucked blood feathers was the source of DNA used in genetic sex analyses.

We sampled a total of 253 chicks, representing more than 90% of fledged offspring of all nesting territories occupied in the Zapovednik during each year. The data presented in this paper are from the 31 nests at which all chicks were sexed in at least two of the seven years of our study. Only broods in which all chicks were successfully sexed were included in our analyses. We did not consider nests that only produced a single year of offspring because sample size over the long term for territory-level analysis was prohibitively small. DNA extraction and isolation was carried out as described in Rudnick et al. (2005). The sex of each individual was genetically determined by polymerase chain reaction (PCR), using 2550F and 2718R primers (Fridolfsson and Ellegren 1999, Rudnick et al. 2005).

To evaluate temporal variability in offspring sex ratio at territories, we used generalized linear mixed models (GLMMs; SAS v 9.3; PROC GLIMMIX ). Our models evaluated the number of male chicks as a proportion of total chicks, with a binomial response and a logit link function (Krackow and Tkadlec 2001; Wojczulanis-Jakubas et al. 2013).  We included a unique brood identifier as a random effect.

Our GLMM tested for spatial and temporal population level differences in offspring sex ratio, and included breeding region and year as categorical fixed effects. Although territory density was not included directly in this model, since our analysis considers two relatively high-density areas (SN and T) and two relatively low density areas, (NN and S), comparison among the two  should reveal density-driven effects.

To evaluate deviance from parity in broad-scale regional pooled data, we used a log-likelihood ratio goodness of fit test (G-statistic) with a Yates correction (Zar 1999; McDonald 2009). In this context, the value of the G-statistic is a measure of skew of sex ratios, with high values indicating high degrees of skew (either male or female biased). In these analyses, following grouping schemes aimed at identifying broad patterns (e.g., Juola and Dearborn, 2007), we did not use the territory as the sampling unit. In our initial characterization of sex ratio we summed the numbers of chicks of each sex produced reserve-wide (hereafter, "reserve-wide sex ratios"). In years where reserve wide patterns suggested a significant deviation from parity, we then repeated this test, summing chicks within each of the four regions within the reserve (hereafter, "regional sex ratios"). This pooling approach allows inference about regional level patterns in sex ratios of offspring

distinct from that provided by the GLMM (above), but it does not permit inference about the adaptive response of individual pairs of eagles (Hurlbert 1984).

**Results.**

Of the 253 chicks sampled during the study period, we genetically determined the sexes of 239. Of these 239 individuals, 123 were male and 116 were female. Twenty chicks came from nests where not all chicks were successfully sampled or from territories with less than two years of data, and thus only 219 chicks (115 males, 104 females) from 119 broods at 30 territories were used for analysis.

We monitored between 9 (2004) and 27 (2000) territories and average sex ratio at all territories ranged from 0.31 (2004) to 0.77 (2001) (Table 1). There was no effect of region, and by extension of territory density, on territory level brood sex ratio (GLMM: $F_{3,108} = 0.59$, $P = 0.6256$) and year was significant only at the $\alpha = 0.10$ level (GLMM: $F_{6,108} = 1.89$, $P = 0.0894$).

Annual reserve-wide sex ratio averaged $0.52 \pm 0.54$ ($\pm$SE) and ranged from 0.33 in 2004 to 0.76 in 2001 (Table 3a). The number of chicks considered ranged from 15 in 2004 to 55 in 2000. When data from all years were considered together, we found no significant deviation from parity in reserve-wide sex ratio (Table 2a; $N = 209$).

There was only one year (2001) in which reserve-wide sex ratio deviated significantly from parity (BSR = 0.76; Table 2a). In that year there was a general trend towards male-bias in chick sex ratios in three regions (Sip-sin [BSR = 0.8], Tersec [0.91], south Naurzum [0.83]) but the deviation from parity was statistically significant only in Tersec (Table 2b).

**Discussion.**

Management of protected areas has impacts for the species in those areas. Likewise, theory predicts that under certain circumstances there will be a selective advantage to skewing offspring sex ratios. Few patterns of the patterns we observed in offspring sex ratio of Eastern Imperial Eagles were linked to environmental variation in a way that either supported or undermined established theory. Furthermore, because we were not able to observe sex ratio at hatching our data provides insight into the outcome of manipulations that may have occurred but limited information on potential mechanisms that resulted in that outcome (i.e., limited information to evaluate primary versus secondary manipulation).

We observed only one case where offspring sex ratios consistently varied significantly from parity (Tersec in 2001, 10 males:1 female; Table 1). Though year was a statistically significant effect in our GLMM only at the $\alpha = 0.10$ level, the model suggests that 2001 was an exceptional year (Table 1), corroborating the more broadly-based aggregated data that are suggestive of trends towards producing males (Table 3). Small sample size notwithstanding, we observed few regional differences in brood sex ratio. We interpret the lack of a significant region effect on sex ratio variation as suggesting either that: 1) eagles in all regions and at all territory densities respond similarly to two large-scale putative sex ratio skewing stimuli (temperature and precipitation) regardless of differences in diet and productivity; 2) Eastern Imperial Eagles at Naurzum do not regularly manipulate the sex ratio of their broods in response to the spatially scaled stimuli that we measured. Since we know that eagles do show different demography in different regions (Katzner et al. 2005, 2006a), the first explanation seems unlikely. The great deal of intra-regional and intra-annual variation observed in chick sex ratio indicated that the second possibility is more likely.

Our analyses provide little evidence that chick sex ratios of Imperial Eagles were skewed, across years or in response to the dietary or environmental variability we measured. However, the one regional statistically significant deviation from sex ratio parity in our study (Tersec in 2001) hints at the possibility that irregular events may have consequence to eagle demography. Because these studied occurred in a protected area, they highlight the importance of protecting enough space to allow for within-population variability to play out naturally, thus ensuring persistence of naturally variable populations.

**Table 1.** *Annual observed sex ratios and modelled solutions for fixed effects (on a logit scale) and intercept value for spatial (region) and temporal (year) terms of a generalized linear mixed model for effects of year and region on brood sex ratio at territories of Eastern Imperial Eagles at the NaurzumZapovednik, Kazakhstan, from 1998-2004. Regional observed values are grand means ± SE, annual means are ± SD. DF = 108 for all comparisons of effect estimates against the reference value.*

| Model Term | N | Mean brood sex ratio (±SD/SE) | Effect estimate (±SE) | t | P |
|---|---|---|---|---|---|
| Intercept | | | $-0.62 \pm 0.59$ | -1.05 | 0.2949 |
| 1998 | 13 | $0.65 \pm 0.47$ | $1.42 \pm 0.76$ | 1.88 | 0.0634 |
| 1999 | 18 | $0.47 \pm 0.41$ | $0.71 \pm 0.65$ | 1.10 | 0.2742 |
| 2000 | 27 | $0.57 \pm 0.36$ | $0.92 \pm 0.61$ | 1.50 | 0.1354 |
| 2001 | 16 | $0.77 \pm 0.35$ | $1.89 \pm 0.70$ | 2.68 | 0.0085 |
| 2002 | 21 | $0.44 \pm 0.39$ | $0.58 \pm 0.65$ | 0.90 | 0.3714 |
| 2003 | 14 | $0.43 \pm 0.42$ | $0.44 \pm 0.67$ | 0.65 | 0.5168 |
| 2004 | 9 | $0.31 \pm 0.43$ | 0 | -- | -- |
| S. Naurzum | 7 | $0.51 \pm 0.28$ | $-0.27 \pm 0.36$ | -0.76 | 0.4464 |
| N. Naurzum | 7 | $0.46 \pm 0.26$ | $-0.35 \pm 0.39$ | -0.89 | 0.3736 |
| Sip-spin | 7 | $0.55 \pm 0.21$ | $0.16 \pm 0.44$ | 0.37 | 0.7113 |
| Tersec | 7 | $0.52 \pm 0.24$ | 0 | -- | -- |

**Table 2.** *(a) Reserve-wide sex ratio variation of Eastern Imperial Eagle chicks at the Naurzum Zapovednik, Kazakhstan. Data are pooled across nesting territories and regions. (b) Regional level (NN = North Naurzum; C = Sip-sin; SN = South Naurzum; T = Tersec) sex ratios of Eastern Imperial Eagle chicks at the NaurzumZapovednik, Kazakhstan from 2001. Data are pooled across nesting territories within each region.*

| | Year | Biotic Scale | n chicks | Sex ratio | G (Yates corrected) | P (2-tailed, df = 1) |
|---|---|---|---|---|---|---|
| (a) | 1998 | Reserve-wide | 17 | 0.65 | 0.95 | 0.3297 |
| | 1999 | Reserve-wide | 35 | 0.49 | 0 | 1 |
| | 2000 | Reserve-wide | 57 | 0.54 | 0.28 | 0.5961 |
| | 2001 | Reserve-wide | 29 | 0.76 | 7.05 | 0.0078 |
| | 2002 | Reserve-wide | 38 | 0.45 | 0.24 | 0.6263 |
| | 2003 | Reserve-wide | 28 | 0.43 | 0.32 | 0.5703 |
| | 2004 | Reserve-wide | 15 | 0.33 | 1.08 | 0.2988 |
| | 1998-2004 | Reserve-wide | 219 | 0.53 | 0.46 | 0.4991 |
| (b) | 2001 | Regional - NN | 7 | 0.43 | 0 | 1 |
| | 2001 | Regional – C | 5 | 0.8 | 0.82 | 0.3644 |
| | 2001 | Regional – SN | 6 | 0.83 | 1.57 | 0.2102 |
| | 2001 | Regional - T | 11 | 0.91 | 6.49 | 0.0108 |

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# ВОССТАНОВЛЕНИЕ БУХАРСКОГО ОЛЕНЯ В РЕСПУБЛИКЕ КАЗАХСТАН – КОМПОНЕНТ КОМПЛЕКСНОЙ ПРОГРАММЫ ВОССТАНОВЛЕНИЯ ВИДА В ЦЕНТРАЛЬНОЙ АЗИИ

## BUKHARA DEER RESTORATION IN KAZAKHSTAN – A COMPONENT OF THE REGIONAL INTEGRATED PROGRAMME OF THE SPECIES CONSERVATION AND RESTORATION IN CENTRAL ASIA

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Бухарский олень *(Cervuselaphusbactrianus*Lydd) считается одним из достаточно много-численных подвидов благородного оленя, хотя фактически с момента его описания отмечалась специфика морфологии и экологии этой формы [5, 18]. Это один из самых мелких благородных оленей, со значительно меньшим половым диморфизмом веса и размера тела; структура и размеры рогов бухарского оленя выделяют его в особую группу, близкую к древним формам оленей, которая дала начало двум группам подвидов – западная группа европейских благородных оленей и восточная группа сибирских (марал, изюбрь) и американских форм. В настоящее время бухарский олень в Международной Красной книге, и необходимость его охраны рассматривается только на уровне подвида благородного оленя (не угрожаемого на уровне вида в целом); при этом он внесен в Приложение II СИТЕС (как *C. e.*