Ornis Fennica 85:46–54. 2008

Parental testosterone and estradiol concentrations in the early nestling period correlate with the age-dependent breeding performance in Tawny Owls *Strix aluco*

Lajos Sasvári, Péter Péczely & Zoltán Hegyi

Sasvári, L.: Eszterházy Károly College of Education, Department of Zoology, H-3300 Eger, Leányka u. 6. Hungary. lsasvari69@yahoo.com (corresponding author)
Péczely, P.: Szent István University, Department of Reproductive Biology, H-2103 Gödöllő, Hungary
Hegyi, Z.: Management of Duna-Ipoly National Park, H-1021 Budapest, Hűvösvölgyi út 52, Hungary

Received 14 June 2007, revised 19 December 2007, accepted 19 December 2007

Steroid hormones, which have not previously been examined in nocturnal raptors, were studied in male and female Tawny Owls (*Strix aluco*). The role of sex steroids during their peak concentrations – the territorial period and egg formation – has recently been documented. During post-hatching periods, the breeding success might negatively correlate with steroid levels. To test this idea, we analysed testosterone and estradiol concentrations during the early nestling period of Tawny Owl, and linked the hormone levels to parental age and breeding performance. We found that females older than two years laid more eggs with higher hatching success than did younger females, and pairs with males older than two years achieved higher fledging success than did pairs with younger males. Testosterone levels in males and testosterone and estradiol levels in females were higher in three-years old than in younger individuals. In females, estradiol and testosterone concentrations positively correlated with the number of eggs laid and their hatching success. In males, testosterone concentrations positively correlated with fledging success. These findings suggest that parents with higher hormone levels in early nestling period are of higher quality with respect to breeding performance.

1. Introduction

Temporal variation in the plasma concentrations of sex steroids are regulated by several factors, including photoperiod and food availability (Wingfield 1983). Outside the breeding season, the plasma concentrations of testosterone are typically low, rise considerably when breeding territories are established, drop rapidly during the incubation, and are low during the nestling period (Ball & Wingfield 1987, Vleck & Brown 1999, Wada *et al.* 1999). An increase in parental effort with decreasing testosterone levels has been shown for songbirds (Hegner & Wingfield 1987, Dittami *et al.* 1991, Beletsky *et al.* 1995, Ketterson *et al.* 1996) and sandpipers (Oring *et al.* 1989). Estradiol levels in early breeding period may be high in Kiwi males (Cockrem & Potter 1991), penguins (Fowler *et al.* 1994), herons (Janz & Bellward 1997) and songbirds (Marler *et al.* 1988). Testosterone and estradiol concentrations of free-living females may be high in the egg-formation and laying periods, and decline during incubation (Cockrem & Potter 1991, Wingfield & Farner 1993, Fowler *et al.* 1994).

Studies investigating the impact of plasma steroids on the breeding success have demonstrated their effects on sexually-selected traits (Borgia & Wingfield 1991, Wingfield & Farner 1993, Poesel *et al.* 2001, Foerster & Kempenaers 2005). However, the relationships between steroid hormone levels and reproductive success have not previously been examined in nocturnal raptors. Hence we conducted a study on the relationship between hormone (plasma testosterone and estradiol) levels and breeding performance of Tawny Owls (*Strix aluco*). The role of sex steroids on breeding performance has previously been based on hormone analysis carried out during the territorial and mating period, when both testosterone and estradiol are at their peak levels (Wingfield & Farner 1993, Poesel *et al.* 2001, Foerster & Kempenaers 2005). Our aim was to address whether, in the low-steroid-level nestling season, there is a relationship between hormone concentrations and breeding success of parents.

Hormone levels have been shown to increase with age in Red-winged Blackbirds *Agelaius phoeniceus* (Beletsky *et al.* 1989), White-crowned Sparrows *Zonotrichia leucophrys* (Morton *et al.* 1990) and Dark-eyed Juncos *Junco hyemalis* (Enstrom *et al.* 1997). Long-term studies on owls have documented a positive correlation between parent age and their breeding performance (Korpimäki 1988, Gehlbach 1989, Saurola 1989). On the basis of these findings, we predicted age-dependent relationships between hormone concentrations and breeding success. Thus, we compared younger (≤2 years old) and older (>2 years old) parents.

The Tawny Owl is a strongly monogamous resident species in Central Europe. Males feed females during the incubation period and, in early nestling period when females brood the chicks, both females and owlets. We expected that parental care provided by females and males would have different effects on offspring survival. Hence, we analysed age-dependent breeding performance separately for female and male adults.

The impact of weather conditions on the reproductive performance of Tawny Owls varies between years (Sasvári & Nishiumi 2005, Sasvári & Hegyi 2005). Parents raise fewer fledglings in years with extensive snow cover during the early nestling period than in years without snow cover. Hence, we predicted that the testosterone and estradiol concentrations sampled in snowy years would be higher than the concentrations measured in years without snow cover.

2. Material and methods

From 1992 to 2003, nest boxes ($n = 220$) for breeding Tawny Owls were placed in a mixed oak/hornbeam/beech (*Quercus cerris*, *Carpinus betulus* and *Fagus sylvatica*) forest in the Pilis Biosphere Reserve located 30 km north-west of Budapest, Hungary (47°35'N; 19°02'E). Six to eight nest boxes were grouped together 300–600 m apart. Groups were separated by 2–5 km. Nest boxes were checked at 4–8 day intervals from the beginning of the egg-laying period (first week in February) until last chicks fledged (in June). For each clutch, we recorded the number of eggs, hatching success, fledging success, and number of fledglings. Hatching success and fledging success were calculated as the number of eggs hatched per number of eggs laid, and the number of nestlings fledged per number of nestlings hatched, respectively.

In early nestling period when owlets were 3–5 days old, adults were captured by placing a net over the nest box entrance between 18.00–22.00 when one or both parents were inside the box. Adults were ringed using different combinations of coloured rings to permit individual identification. Age determination was based on the moult of primaries and secondaries (Petty 1992). Young and old (experienced) parents were distinguished as being ≤ 2 and >2 years old.

We selected 51 pairs of Tawny Owls (3–6 pairs each year berween 1992–2003) for the analysis of testosterone and estradiol concentrations and for the examination of breeding performance. Based on the age of each member of a pair, we categorized pairs as follows: (1) both adults were ≤ 2 years old, (2) the male was > 2 years old and the female ≤ 2 years old, (3) the female was > 2 years old and the male ≤ 2, (4) both adults were > 2 years old. Different individuals were studied for all

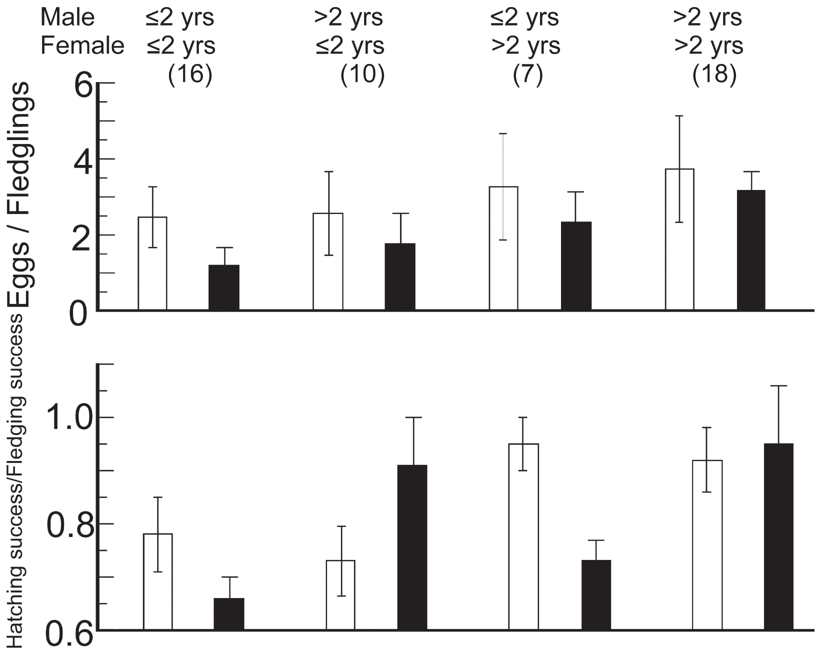

Fig. 1. Mean (± SD) number of eggs, hatching success, fledging success, and number of fledglings for Tawny Owl pairs with males and females of different ages. White columns indicate the number of eggs and hatching success, and black columns indicate the number of fledglings and fledging success. Number of pairs in parentheses.

breeding pairs, and same pairs were not followed in subsequent years.

Between-year variation in the hormone levels of males and females were examined in terms of cold (extensive snow cover in the early nestling period) versus mild (no or little snow) weather conditions. Years with complete snow cover within a 1-km radius from a given nest box during the egg-laying period, incubation and early nestling period (until 10 days after the hatching of the last young) were considered to be ‘cold’breeding year for any given pair.

Blood samples were obtained from adults by applying brachial venipuncture within 15 min of capture. Blood was drawn into heparinized polyethylene tubes and stored in a cooler. After transportation to the lab and centrifugation, the plasma was immediately stored at –20°C. Plasma samples (20–100ml) were extracted with 10 volumes of diethylether, three times (30min extraction time). Testosterone concentrations were analysed, without chromatography, using duplicate radioimmunoassay (RIA; Péczely *et al.* 1980). The detection limit was 5 pg/ml. The coefficients of variation showed 8% and 11% intra- and inter-assay differences, respectively. The antibody cross-reacts with 5-α-dihydrotestosterone at a level of 40%, but as the known concentration of this androgen in the avian plasma is generally <10% of testosterone, it is likely to affect measured testosterone concentration only slightly (Péczely & Pethes 1979).

Estradiol was determined using an antiserum raised in sheep to estradiol-17β-hemisuccinate conjugated to BSA (Bovine Serum Albumin) through the 17 position, at a final dilution of 1:50,000, that gave a maximum binding of 40%. In this case, there was 30% cross reactivity with estrone. Inter- and intra-assay precision were 8% and 10% for estradiol as expressed as coefficients of variation at minimum detectable concentrations. The sensitivity of assay was 10–15 pg/ml of steroid.

Statistical analyses were performed using the SPSS statistical package (Norusis 1994). A two-way ANOVA was used to evaluate if the breeding performance was affected by the age of pairs. A multiple regression analysis was performed to determine if parental age or male and female testosterone and estradiol levels affected the breeding performance. The effects of within-pair age composition on the reproductive performance in different breeding periods were tested using the GT2 method for multiple comparisons (unplanned comparisons among means for unequal sample sizes; Sokal & Rohlf 1980).

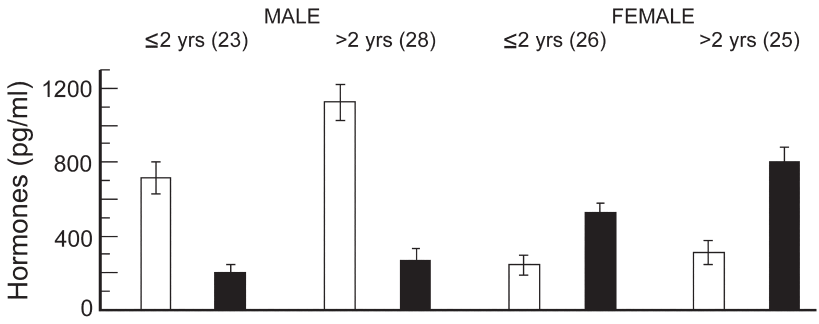

Fig. 2. Plasma testosterone and estradiol concentration (pg/ml ± SD) of male and female Tawny Owls in relation to their age. White and black columns indicate testosterone and estradiol concentrations, respectively. Number of parents shown in parentheses.

Table 1. Two-way ANOVA for the effect of parental age on the breeding performance of Tawny Owls. Young, i.e., ≤ 2 years old and old, i.e., > 2 years old individuals were compared.

		Number of eggs	Hatching success	Fledging success	Number of fledglings
Age of males (n = 51, df = 2)	F	4.01	3.98	8.03	6.13
	P	0.053	0.059	0.007	0.019
Age of females (n = 51, df = 2)	F	8.65	7.18	4.09	4.88
	P	0.005	0.009	0.047	0.036
Interactions (df = 2)	F	4.86	4.21	5.07	5.37
	P	0.037	0.043	0.030	0.024

3. Results

Within-pair age differences influenced the breeding performance (Fig. 1; Table 1). Pairs with both adults £ 2 years old had the smallest clutches, fewest fledglings, and lowest fledging success, as compared with pairs with both adults > 2 years old that had, on average, the largest clutches, most fledglings, and highest fledging success. Pairs with a young female and an old male had low hatching success, whereas pairs consisting of an old female and a young male tended to have high hatching success.

The effect of the age of males and females on the reproductive performance was different between pre- and post-hatching seasons (Table 2). Pairs with females > 2 years old had larger clutches and greater hatching success than pairs with younger females, but the age of males did not significantly influence the reproductive processes at these stages of the breeding period. Moreover, pairs with males > 2 years old had greater fledging

Table 2. Multiple comparisons (GT2 method) among the number of eggs, hatching success and fledging success produced by Tawny Owl pairs of different age combinations. Numbers in parentheses indicate four categories of parent age composition: (1) both parents ≤ 2 years old; (2) male > 2 years old, female ≤ 2 years old; (3) male ≤ 2 years old, female > 2 years old; (4) both parents > 2 years old. Numerical values with two decimal digits indicate differences in absolute values between groups (minimum significant difference, MSD); asterisks indicate significant differences at the 0.05 level.

	Number of eggs				Hatching success				Fledging success			
	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)
(1)	–	0.42	0.65	0.78	–	0.03	0.08	0.09	–	0.14	0.16	0.13
(2)	0.29	–	0.25	0.47	0.05	–	0.05	0.07	0.25*	–	0.24	0.09
(3)	0.81*	0.43*	–	0.33	0.16*	0.10*	–	0.08	0.10	0.33*	–	0.20
(4)	0.87*	0.60*	0.18	–	0.21*	0.13*	0.03	–	0.21*	0.04	0.31*	–

Table 3. Two-way ANOVA for the effect of parental age and weather on testosterone and estradiol concentration of male and female Tawny Owls. “Parental age” classes were ≤ 2 years old and > 2 years old; “Weather” indicates the similarity between snowy years and years without snow cover during the early nestling period.

		Males (n = 51)		Females (n = 51)	
		Testosterone	Estradiol	Testosterone	Estradiol
Parental age (df = 2)	F	5.39	3.36	4.97	7.92
	P	0.027	0.071	0.039	0.007
Weather (df = 2)	F	3.24	3.19	3.40	6.89
	P	0.080	0.083	0.076	0.012
Interactions (df = 2)	F	3.95	3.39	3.82	7.21
	P	0.053	0.077	0.051	0.009

Table 4. Multiple regression coefficients (B) between breeding performance, parental age and testosterone and estradiol concentrations of Tawny Owls. * = P < 0.05; ** = P < 0.01; *** = P < 0.001; for all tests, n = 51.

	Males				Females			
	Age	Testosterone	Estradiol	R^2	Age	Testosterone	Estradiol	R^2
Number of eggs	0.091	0.083	0.011	0.084	0.240***	0.217*	0.297***	0.269
Hatching success	0.070	0.009	0.005	0.060	0.192**	0.071*	0.133**	0.125
Fledging success	0.214***	0.138***	0.007	0.194	0.088	0.0088	0.006	0.046
Number of fledglings	0.170**	0.110**	0.009	0.113	0.142*	0.044*	0.109*	0.092

success than pairs with younger males. Female age, however, did not have such an effect during the nestling period.

Older males (> 2 years old) had higher testosterone concentrations than had younger males (£ 2 years old; t_{50} = 14.46, P < 0.001), but estradiol concentrations did not show a significant tendency with age (t_{50} = 1.87, P = 0.069; Fig. 2). Similarly, older females had higher testosterone and estradiol concentrations than had younger females (t_{50} = 2.65, P = 0.012 and t_{50} = 9.30, P < 0.001, respectively). Estradiol and testosterone levels were positively correlated in females (r = 0.39, P = 0.004, n = 51), but not in males (r = 0.09, P = 0.114, n = 51).

Neither males nor females showed differences in testosterone levels between snowy years and years without snow cover (males: 935.2 ± 52.4 pg and 952.3 ± 47.7 pg; t_{50} = 1.21, P = 0.273; females: 197.4 ± 21.4 pg and 217.0 ± 40.6 pg; t_{50} = 1.44, P = 0.160). Females had lower estradiol levels in cold than in mild weather conditions (596.1 ± 63.5 pg and 683.0 ± 70.2 pg; t_{50} = 5.90, P < 0.001), but estradiol concentrations were not significantly affected by weather in males (201.2 ± 29.3 pg and 217.7 ± 35.6 pg; t_{50} = 1.86, P = 0.077). Two-way ANOVA revealed interactions between the effects of parental age and weather conditions only on the estradiol concentration in females (Table 3).

Male testosterone concentration and male age significantly and positively affected the fledging success and number of fledglings (Table 4). In females, both the testosterone and estradiol levels and age positively correlated with the number of eggs and fledglings, and with hatching success. Fledging success, however, was not influenced by the relationship between female hormones and age. Presumably the positive correlations with the number of fledglings reflected the relationships between hormone levels and breeding performances at earlier stages of the reproductive process.

4. Discussion

We showed a correlation between sex steroid levels and age-dependent breeding performance in Tawny Owls. Our findings may be summarised as follows: (1) analyses of testosterone and estradiol concentrations in Tawny Owl parents during the nestling period showed that sex steroids may be linked to breeding performance during the provisioning phase, i.e., when the hormone levels are low. (2) The relationship between hormone levels and breeding success was age-dependent: older (i.e., >2 years old) males having higher testosterone levels and older females having higher estradiol and testosterone concentrations produced more fledglings than younger birds with lower hormone levels. (3) Female and male age had distinct effects at different stages of the breeding season: older females laid more eggs with higher hatching success than younger females, while pairs with males older than two years achieved a higher breeding success.

The age-related steroid concentrations in Tawny Owls do not indicate a causal link between age and hormone levels. We did not conduct longitudinal examinations on the same parents; hence, the higher concentrations recorded in older parents might be due to a lower mortality as compared with young owls with low steroid levels. Nevertheless, a possible explanation for a link between the increased reproductive success of older owls and hormone concentrations may be based on the relationship between the ability to defend a territory and the levels of androgens. Tawny Owl males occupy territories of various quality in terms of prey availability (Redpath 1995, Ranazzi *et al.* 2000, Francis & Saurola 2004, Solonen 2005). We have shown earlier that Tawny Owls with a breeding experience of 3–4 years occupied better-quality territories with rich prey resources as compared with less experienced breeders (Sasvári *et al.* 2000, Sasvári & Hegyi 2005). They also fed more frequently and reached a higher reproductive performance than did younger parents (Sasvári *et al.* 2000, Sasvári & Hegyi 2005). High testosterone levels in males are related to agression (Beletsky *et al.* 1990), intense vocalization activity (Ketterson *et al.* 1992, Hunt *et al.* 1997) and occupation of high-quality territories (Silverin 1980, Wingfield 1984, Chandler *et al.* 1994). Despite the drop during incubation, the difference in concentration levels continues to reflect differences in the quality of males even during the nestling period and the increased fledging success of high-testosterone males may be due to the acquisition of a high-quality territory, rich in prey, during the early breeding season.

We found that older females had higher levels of testosterone and estradiol levels than had younger females, although there were no age-related differences in male estradiol levels. Compared with young females, older females had higher estradiol and testosterone concentrations, produced more eggs laid and had greater hatching success. Estradiol levels have been reported to rise as follicles grow before the egg laying in free-living birds (Cockrem & Potter 1991, Wingfield & Farner 1993), and low fecundity of young females, compared to older females has been documented both in passerines (Perrins 1979, Dhondt 1989) and non-passerines (Newton 1989, Sydeman *et al.* 1991). Concentrations of testosterone were lower than those of estradiol, but higher testosterone levels were still related to a higher breeding performance in female Tawny Owls.

A relationship between weather conditions and hormone concentrations was revealed only for females: higher estradiol levels were recorded in mild years without snow cover than in snowy years. High clutch size links high estradiol levels in females of several bird species (Wingfield & Farner 1993), and we found that Tawny Owls laid more eggs in mild than in cold years, the temperature being related to the harshness of weather conditions (Sasvári & Hegyi 2005). Perhaps the high estradiol levels, measured in the post-hatching period in the present study, might be due to the ability of females to maintain hormone levels at the high levels typical for mild years.

Like virtually every species examined to date (Cockrem & Potter 1991, Fowler *et al.* 1994, Janz & Bellward 1997, Hirschenhauser *et al.* 1999, Washburn *et al.* 2004), male Tawny Owls have higher testosterone and lower estradiol levels than females. In conclusion, the observed relationship between hormone levels and breeding performance, would suggest that the testosterone concentration in males, and both the testosterone and estradiol concentration in females, indicates the quality of parents. As the organizational and activational effects of reproductive steroid hormones are greater in the early stage of breeding period, both the testosterone and estradiol might strongly reflect the breeding ability of parents. Nevertheless, the relationship between hormone levels and parental quality was also detectable in the late stage of the reproductive period of Tawny Owls.

On the basis of this correlative study, causal conclusions between the steroid hormones and breeding performance can be stated only with caution. Insight into the hormonal influence on Tawny Owl breeding processes would require an analysis of circulating levels of plasma steroids. In Central Europe, male competition for territories takes place in autumn, while females lay eggs at the end of winter and in early spring. As there is a long interval between the acquisition of territory and the provisioning period unique relationships between hormone levels and the different stages of the breeding process may exist in Tawny Owls. Future experiments could examine interactions between male territorial activity and prey supply for nestlings, related to parental hormone concentrations.

Acknowledgements. We are indebted to anonymous referees for their constructive comments and suggestions. We are also grateful to Susan Totterdell, Department of Pharmacology, University of Oxford, for linguistic corrections. This work was supported by the Department of Zoology of Eszterházy Károly College, Eger, and Hungarian National Foundation for Scientific Research (project number: T 067669). We are also grateful to the Frank M. Chapman Memorial Fund, The American Museum of Natural History, for financial support. All research was carried out in accordance with the legal and ethical standards of the country in which the work was performed.

Pesäpoikasajan emolintujen elimistön testosteroni- ja estradiolipitoisuudet korreloivat iästä riippuvaisen pesimämenestyksen kanssa lehtopöllöllä *Strix aluco*

Tutkimme lehtopöllön steroidihormoneja, aihe, jota ei ole yöaktiivisilla petolinnuilla aiemmin tarkasteltu. Sukupuolisteroidien merkitys on hiljattain todettu reviirinmuodostuksen ja munien kehittymisen aikana. Kuoriutumisen jälkeinen pesimämenestys saattaa korreloida negatiivisesti steroiditason kanssa. Selvittääksemme kysymystä analysoimme lehtopöllöjen testosteroni- ja estradiolipitoisuuksia pesimäkauden alkupuolella ja pyrimme yhdistämään hormonitasot emolinnun ikään ja pesimämenestykseen.

Havaitsimme, että yli kaksivuotiaat naaraat munivat useampia munia paremmalla kuoriutumismenestyksellä kuin nuoremmat naaraat, ja parit, joiden koiras oli yksi kaksivuotias, tuottivat enemmän lentopoikasia kuin parit, joiden koiras oli nuorempi. Testosteronitasot koirailla ja testosteroni- ja estradiolitasot naarailla olivat korkeampia kolmevuotiailla kuin nuoremmilla yksilöillä. Naaraiden testosteroni- ja estradiolipitoisuudet korreloivat positiivisesti munittujen munien määrän ja niiden kuoriutumisprosentin kanssa. Koirailla testosteronipitoisuus korreloi positiivisesti parin tuottaman lentopoikasten määrän kanssa. Havainnot viittaavat siihen, että parit, joiden hormonitasot ovat varhaispesimäkaudella korkeampia ovat pesimämenestyksen suhteen tarkasteltuna parempilaatuisia.

References

Ball, G. F. & Wingfield, J. C. 1987: Changes in plasma levels of luteinizing hormone and sex steroid hormones in relation to multiple-broodedness and nest-site density in male starlings. — Physiology and Zoology 60: 191–199.

Beletsky, L. D., Orians, G. H. & Wingfield, J. C. 1990: Effects of exogenous androgen and antiandrogen on territorial and nonterritorial red-winged blackbirds (Aves: Icterinae). — Ethology 85: 58–72.

Beletsky, L. D., Gori, D. F., Freeman, S. & Wingfield, J. C. 1995: Testosterone and polygyny in birds. — In Current Ornithology Vol. 8 (ed. Power, D. M.). Plenum, New York.

Beletsky, L. D., Orians, G. H. & Wingfield, J. C. 1989: Relationships of steroid hormones and polygyny to territorial status, breeding experience and reproductive success in male Red-winged Blackbirds. — Auk 106: 107–117.

Borgia, G. & Wingfield, J. C. 1991: Hormonal correlates of bower decoration and sexual display in the satin bowerbird. — The Condor 93: 935–942.

Chandler, C. R., Ketterson, E. D., Nolan, V. Jr & Ziegenfus, C. 1994: Effects of testosterone on spatial activity in free-ranging male dark-eyed juncos, *Junco hyemalis*. — Animal Behaviour 47: 1445–1455.

Cockrem, J.F. & Potter, M. A. 1991: Reproductive endocrinology of the North Island Brown Kiwi *Apteryx australis mantelli*. — In Symposium of Endocrinology of Avian Breeding Systems. Acta XX Congressus

Internationalis Ornithologici: 2092–2101. Christchurch, New Zealand.

Dhondt, A. A. 1989: The effect of old age on the reproduction of Great Tits *Parus major* and Blue tits *P. caeruleus*. — Ibis 131: 268–280.

Dittami, J., Hoi H. & Sageder, G. 1991: Parental investment and territorial/sexual behavior in male and female reed warblers: are they mutually exclusive? — Ethology 88: 249–255.

Enstrom, D. A., Ketterson, E. D. & Nolan, V. Jr. 1997: Testosterone and mate choice in the dark-eyed junco. — Animal Behaviour 54: 1135–1146.

Foerster, K. & Kempenaers, B. 2005: Effects of testosterone on male-male competition and male-female interactions in blue tits. — Behavior Ecology and Sociobiology 27: 215–223.

Fowler, G. S., Wingfield, J. C., Boersma, P. D, & Sosa, R. A. 1994: Reproductive endocrinology and weight change in relation to reproductive success in the Magellanic Penguin (*Spheniscus magellanicus*). — General and Comparative Endocrinology 94: 305–315.

Francis, C. M. & Saurola, P. 2004: Estimating components of variance in demographic parameters of Tawny Owls *Strix aluco*. — Animal Biodiversity and Conservation 27: 489–502.

Gehlbach, F.R. 1989: Screech-Owl. — In: Lifetime Reproduction in Birds. (ed. Newton, I.): 315–326. Academic Press, London.

Hegner, R. E. & Wingfield, J. C. 1987: Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. — Auk 104: 470–480.

Hirschenhauser, K., Möstl, E. & Kotrschal, K. 1999: Within-pair testosterone covariation and reproductive output in Greylag Geese *Anser anser*. — Ibis 141: 577–586.

Hunt, K. E., Hahn, T. P. & Wingfield, J. C. 1997: Testosterone implants increase song but not aggression in male Lapland Longspurs. — Animal Behaviour 54: 1177–1192.

Janz, D. M. & Bellward, G. D. 1997: Effects of acute 2,3,7,8-tetrachlorodibenzo-P-dioxin exposure on plasma thyroid and sex steroid hormone concentrations and estrogen receptor levels in adult great blue herons. — Environmental Toxicology and Chemistry 16: 985–989.

Ketterson, E. D., Nolan, V. Jr., Cawthorn, M. J., Parker, P. G. & Ziegenfus, C. 1996: Phenotypic engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. — Ibis 138: 70–86.

Ketterson, E. D., Nolan, V., Wolf, L. & Ziegenfus, C. 1992: Testostrone and avian life histories: effects of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed Junco (*Junco hyemalis*). — American Naturalist 140: 980–999.

Korpimäki, E. 1988: Effects of age on breeding performance of Tengmalm's Owl *Aegolius funereus* in western Finland. — Ornis Scandinavica 19: 21–26.

Marler, P., Peters, S., Ball, G. S., Dufty, A. M. Jr. & J. C. Wingfield. J. C. 1988: The role of sex steroids in the aquisition and production of birdsong. — Nature 336: 770–772.

Morton, M. L., Peterson, L. E., Burns, D. M. & Allan, N. 1990: Seasonal and age-related changes in plasma testosterone levels in mountain white-crowned sparrows. — The Condor 92: 166–173.

Newton, I. 1989: Lifetime Reproduction in Birds. — Academic Press, London, UK.

Norusis, M. 1994: SPSS Advanced Statistics 6.1. — SPSS Inc., Chicago.

Oring, L. W., Fivizzani, A. J. & El Halawani, M. E. 1989: Testosterone-induced inhibition of incubation in the spotted sandpiper (*Actitis mecularia*). — Hormones and Behavior 23: 412–423.

Perrins, C. M. 1979: British tits. — Collins, London, England.

Petty, S. J. 1992: A guide to age determination of Tawny Owl *Strix aluco*. — In: The ecology and conservation of European owls (eds. Galbraith, C. A., Taylor, I. R. & Percival, S.): 89–91. Joint Nature Conservation Committee, Peterborough, UK.

Péczely, P. & Pethes, G. 1979: Alterations in plasma sexual steroid concentration in the collared dove (*Streptopelia decaocto*) during sexual maturation and reproductive cícle. — Acta Physiologica, Hungarian Academy of Sciences 54: 161–170.

Péczely, P., Pethes, G. & Rudas, P. 1980: Interrelationship between thyroid and gonadal function in female Japanese quail kept under short and long photoperiods. — Journal of Endocrinology 87: 55–63.

Poesel, A., Foerster, K. & Kempenaers, B. 2001: The dawn song of the blue tit *Parus caeruleus* and its role in sexual selection. — Ethology 107: 521.531.

Ranazzi, L., Manganaro, A. & Salvati, L. 2000: The breeding success of the Tawny Owls *Strix aluco* in a mediterranean area: a long-term study in Rome, Italy. — Journal of Raptor Research 34: 322–326.

Redpath, S. M. 1995: Habitat fragmentation and the individual: Tawny Owls *Strix aluco* in Woodland patches. — Journal of Animal Ecology 64: 652–661.

Sasvári, L., & Hegyi, Z. 2005. Effects of breeding experience on nest-site choice and the reproductive performance of Tawny Owls (*Strix aluco*). — Journal of Raptor Research 39: 26–35.

Sasvári, L., Hegyi, Z., Csörgő, T. & Hahn, I. 2000: Age-dependent diet change, parental care and reproductive cost in tawny owls *Strix aluco.* — Acta Oecologica 21: 267–275.

Sasvári, L., & Nishiumi, I. 2005: Environmental conditions affect offspring sex-ratio variation and adult survival in Tawny Owls. — The Condor 107: 323–328.

Saurola, P. 1989: Ural Owl. — In: Lifetime reproduction in birds (ed. Newton, I.): 327–345. Academic Press, London, UK.

Silverin, B. 1980: Effects of long-acting testosterone treatment on free-living pied flycatchers, *Ficedula hypoleuca*, during the breeding period. — Animal Behaviour 28: 906–912.

Sokal, R. R. & Rohlf, F. J. 1980: Biometry. — W. H. Freeman and Company, San Francisco.

Solonen, T. 2005: Breeding of the Tawny Owl *Strix aluco* in Finland: responses of a southern colonist to the highly variable environment of the North. — Ornis Fennica 82: 97–106.

Sydeman, W. J., Penniman, J. F., Penniman, T. M., Pyle, P. & Ainley, D. G. 1991: Breeding performance in the western gull: effects of parental age, timing of breeding and year in relation to food availability. — Journal of Animal Ecology 60: 135–149.

Vleck, C. M. & Brown, J. L. 1999: Testosterone and social and reproductive behavior in *Aphelocoma* jays. — Animal Behaviour 58: 943–951.

Wada, M., Shimizu, T., Kobayashi, S., Yatani, A, Sandaiji, Y., Ishikawa, T. & Takemure, E. 1999: Behavioral and hormonal basis of polygynous breeding in male bush warblers (*Cettia diphone*). — General Comparative Endocrinology 116: 42–432.

Washburn, B. E., Tempel D. J., Millspaugh, J. J., Gutiérrez, R. J. & Seamans, M. E. 2004: Factors related to fecal estrogens and fecal testosterone in California Spotted Owls. — The Condor 106: 567–579.

Wingfield, J. C. 1983: Environmental and endocrine control of avian reproduction: an ecological approach. — In: Avian endocrinology: Environmental and ecological perspectives (eds. Mikami, S., Homma K. & Wada, M.): 265–288. Springer Verlag, New York.

Wingfield, J. C. 1984: Androgens and mating systems: testosterone-induced polygyny in normally monogamous birds. — Auk 101: 665–671.

Wingfield, J. C. & Farner, D. S. 1993: Endocrinology of reproduction in wild species. — In: Avian Biology (eds. Farner, D. S., King, J. R. & Parkes, K. S.): 164–327. Academic Press, London, UK.