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CAPTIVITY AFFECTS SPERM PRODUCTION, TESTES SIZE AND BEAK COLOR IN HOUSE SPARROWS (*PASSER DOMESTICUS*)

ABSTRACT

We held 17 male House Sparrows in an outdoor aviary from May-July 2005 to repeatedly collect semen samples from them to be used later in the development of artificial insemination techniques. Birds were housed individually, but in visual and auditory contact with other House Sparrows of both sexes. Beginning on 15 June, and every third day thereafter, we used cloacal massage to collect semen samples. By 12 July, only 2/17 (11.8%) of captive males still produced sperm and the beaks of most males had begun changing from black to horn-colored; both are signs of decreased testosterone production. To rule out the possibility that these changes were due to seasonal effects, we captured 9 wild adult male sparrows on 13 July. In contrast to the captive males, most wild sparrows (7/9; 77.8%) produced sperm on 14 July and all of them had completely black beaks. Captive males had significantly less black on their beaks and smaller combined testes mass than did wild males. We found sperm in the testes and seminal glomera of wild but not captive sparrows. These results are consistent with the conclusion that the captive males suffered a decline in testosterone levels with subsequent physiological effects. We hypothesize that the stress of captivity and handling resulted in chronic elevation of corticosterone which caused a decline of testosterone producing testicular atrophy, a decrease in sperm production, and a change in beak color.

Key words – captive studies, House Sparrow, *Passer domesticus*, spermatogenesis, testes, testosterone.

INTRODUCTION

House Sparrows (*Passer domesticus*) are common subjects of both field and laboratory studies of birds because of they are nonmigratory, have a wide geographic range, and live in close association with humans (Lowther and Cink 1992). However, it is unclear why it is difficult to breed these otherwise adaptable birds in captivity (Anderson 2006). The opportunity for social interactions appears to be an important requirement; breeding success is greater in groups of House Sparrows caged together than in isolated pairs (Anderson 2006). Moreover, Hegner and Wingfield (1984) suggested that social facili-

tation may be essential for male gonadal development because they observed greater testicular recrudescence in males housed in flocks than in males housed in pairs. Here we report the results of a study during which we serendipitously found that captivity had a negative effect on sperm production, testes size and beak color in male House Sparrows. This finding may help explain why it is difficult to breed House Sparrows in captivity (Anderson 2006).

We held male House Sparrows in captivity so we could collect multiple semen samples from individual males in an effort to develop artificial insemination techniques (Gee and Sexton 1983, Gee and Temple 1978, Gee et al. 2004) that could eventually be used to study the dynamics of multiple mating in wild birds. We found that sperm production declined in captive males. This was surprising for several reasons. First House Sparrows have been bred, albeit with difficulty, in captivity (Mitchell and Hayes 1973, Baker 1995, Moreno-Rueda and Soler 2002). Second, the failure of non-domesticated wild birds to breed in captivity (Lambrechts et al. 1999, and references therein) is rarely, if ever, attributed to the failure of captive male birds to maintain spermatogenesis (Snyder et al. 1996). Last, in their comprehensive review of artificial insemination techniques in non-domesticated birds Gee et al. (2004) did not report captivity negatively affecting sperm production as a problem typically encountered by aviculturists.

STUDY AREA

We studied House Sparrows that we captured on and within 16 km southeast of the Grand Valley State University (GVSU) campus in Allendale, MI, USA (42.5° 57' N, 85° 53' W).

MATERIALS AND METHODS

We used mist nets and walk-in traps to capture 17 male and eight female sparrows during May-June 2005. When each bird was captured, we measured its mass with a spring scale to the nearest 0.2 g, right tarsus length with an electronic digital caliper to 0.01 mm, and right wing chord length to the nearest 1 mm with a ruler with a stop fixed to one end. We photographed each male's bib (chest area) using a scale bar and a Nikon Coolpix® 5 megapixel digital camera and used SCION image analysis software (www.scioncorp.com) to determine bib area. Each sparrow was assigned an identifying number and given a unique combination of colored plastic bands for individual identification.

Once preliminary measurements were completed, each bird was put into a standard finch breeding cage (33 cm x 27 cm x 33 cm; length x width x height, respectively, with 1.27 cm bar spacing). Each cage contained one hardwood perch, two plastic cups for food and water, and one nest box. Each cage was separated from an adjacent cage by a partition with 1.27 cm bar spacing. Cages stacked on racks so that each rack contained

four sets of two adjacent cages. Consequently, all sparrows were in auditory and visual contact with sparrows of both sexes. Sparrows were housed individually to reduce intraspecific aggression and to reduce the chances of the cloacal microbe transmission between males. This second reason was important because we had also set out to compare the semen microbe loads of individual males. All cages were in a covered screen tent outdoor aviary (9.29 m^2 floor area, 2.44 m center height). The screen tent shaded the sparrows but allowed them to be exposed to natural photoperiods and protected them from rain and biting insects. Nesting materials, food, and water were provided ad libitum. We fed the sparrows a commercial seed mix (KAYTEE Supreme Canary Fortified Daily Blend; crude protein 15%, crude fat 10%, crude fiber 10%, moisture 12%) periodically supplemented with other nutrients and vitamins (Scott's Petamine Breeding Formula) and grit. Cages were cleaned every third day.

Sparrows were held in captivity from 17 May – 14 July. Of the 17 males and eight females, 16 males were individually housed, one male was housed with a female from 31 May – 14 July to determine if pairs housed together would breed in captivity, and the remaining seven females were individually housed. We allowed males to acclimate to the conditions of captivity for at least 4 days before we began to attempt to collect semen samples from them. We restricted the time we spent at the aviary to visits to feed and water birds, clean cages, and obtain semen samples to reduce the amount of investigator-induced stress on the sparrows. In addition, the aviary was located in a partially hidden location on campus not often frequented by people so as to reduce disturbance.

We collected semen samples from captive sparrows every third day from 15 June – 12 July. Samples were collected in 0.5 µL aliquots using cloacal massage (Samour et al. 1986). Lombardo performed all cloacal massages. Because we were attempting to collect sufficient volumes of semen from each male for use in artificial insemination experiments we collected samples until we were unable to obtain any semen during each sampling attempt. Trace samples (samples < 0.5 µL) were collected and recorded as 0.1 µL. Samples were collected using micropipets with sterile tips and diluted 1:1 in Beltsville poultry semen extender (Bakst and Cecil 1977). Sub-samples from this dilution were prepared for cryopreservation and artificial insemination.

Semen samples were examined for the presence of sperm cells the same day they were collected. If sperm cells were present, sub-samples were used to calculate sperm concentrations. Three sub-samples from each sample were counted using an improved Neubauer hemacytometer (Reichert, Buffalo, New York) at 1000X magnification on a compound microscope. We used the mean sperm concentration (sperm μL^{-1}) of the sub-sample counts to calculate the total number of sperm cells obtained in each sample. Our assistant E. McCombs examined samples for sperm and counted sperm in all semen samples.

As sampling progressed, it became increasingly difficult to obtain semen samples. To determine if captivity affected sperm production we compared sperm production of our captive sparrows, hereafter "Captive sparrows," to that of free-ranging sparrows. On 12-13 July, we captured nine adult male House Sparrows, hereafter "Wild sparrows," within a 16 km radius of the GVSU campus; 7/9 were captured in a barn at a dairy farm located less than 16 km northwest of the GVSU campus and the remainder were captured at an apartment complex less than 16 km southeast of campus. We do not know the breeding stages of the males we captured but all of them were in adult breeding plumage and had completely black beaks and there were numerous sparrow nests with nestlings in the rafters of the barn on the dairy farm. Wild sparrows were transported to the GVSU campus, measured, and placed individually into cages as described above and allowed to acclimate to captivity for 24 hr before we attempted to obtain semen samples from them.

On 14 July, we attempted to obtain semen samples from Captive and Wild sparrows and then randomly euthanized 10 Captive and seven Wild sparrows to examine their testes for sperm production and their seminal glomera for stored sperm (King 1981, Wolfson 1954). Sparrows were euthanized by over-anesthetizing them with isoflourane. Within 2 hr of euthanasia, testes were resected and weighed on an electronic balance to 0.001g. We compared the combined testes masses (CTM) of Captive and Wild sparrows. Despite previous evidence of left-right asymmetry in House Sparrow testes mass (Keck 1934), our preliminary analyses showed that there was no significant differences between the masses of left (0.044 ± 0.070 g SD) and right (0.036 ± 0.059 g) testes (paired $t_9 = 2.16$, $P = 0.06$) of either euthanized Captive or Wild sparrows (left testis: 0.354 ± 0.120 g; right testis: 0.339 ± 0.217 g; paired $t_6 = 0.31$, $P = 0.77$). We attempted to resect the seminal glomera from euthanized birds, but they were difficult to find in the Captive sparrows (see below). The left testes from three randomly chosen Captive sparrows and the left testes and seminal glomera from three randomly chosen Wild sparrows were fixed in formalin, embedded in paraffin, sectioned for histological examination, stained, and examined under a microscope for the presence of sperm cells.

Over the course of the sampling period the beaks of male Captive sparrows began to change, starting at the base, from black to horn-colored. Beak color is a secondary sexual characteristic in House Sparrows (Anderson 2006) and it has long been known that beak color in House Sparrows is directly correlated with testosterone levels (T) and testicular activity (Haase 1975, Keck 1933, Keck, 1934, Lofts et al. 1973).

After the birds were euthanized, we photographed a lateral view of each male's beak and used the image analysis software to determine the proportion of the beak that was black. We did this to examine the relationship between beak color and CTM of Captive and Wild sparrows.

We examined the data for normality and, where appropriate, used parametric and nonparametric statistical tests to analyze data using SPSS 10.0 for Windows (SPSS

2002). We removed several data points from correlation analyses when preliminary analyses showed them to be statistical outliers. These included (a) one measure of semen volume (8 μL) that was nearly fifty percent larger than the next largest volume and (b) two measures of sperm concentration that were an order of magnitude larger (107 sperm μL^{-1}) than the next largest sperm concentrations. All tests were two-tailed and differences were considered statistically significant at $P < 0.05$. Data are reported as means ± SD.

RESULTS

To examine the effects of captivity on sperm production we compared the sperm concentrations we obtained from Captive males on 15 June with those obtained on 12 July. The mean sperm concentration of 15 June samples (1,310,124 ± 2,139,669 sperm μL^{-1}) was significantly greater than those collected on 12 July (236,037 ± 969,956 sperm μL^{-1}) (Wilcoxon matched pairs, $z = -1.96$, $P = 0.05$). Furthermore, the sperm concentrations of 15 June samples were larger than those collected on 12 July for 16/17 (94%) of Captive males (sign test, $P = 0.04$). A significantly larger proportion (9/17, 53%) of 15 June samples from Captive sparrows contained sperm cells than did samples collected on 12 July (2/17, 12%) (Fisher exact test, $P = 0.03$). The proportion of semen samples containing sperm cells collected from Captive sparrows on 12 July was significantly smaller than that collected from Wild sparrows (7/9; 78%) on 14 July (Fisher exact test, $P = 0.0016$). Sperm concentrations (Spearman rank correlation, $r_s = -0.40$, $n = 59$, $P = 0.002$) obtained from Captive sparrows declined with the number of days that a male was in captivity.

Over the course of the sampling period the beaks of most Captive sparrows, starting at the base, progressively changed from black to horn-colored. All of the 10 Captive males that were euthanized had completely black beaks at the start of the experiment on 15 June. In contrast, their beaks were significantly less black on 12 July; mean proportion black = 0.74 ± 0.26, (paired – $t_9 = 3.21$, $P = 0.01$). For Captive sparrows, there was not a significant correlation between the proportion black on a beak and semen volume on 14 July ($r = -0.26$, $n = 10$, $P = 0.48$), but those with more black on their beaks had greater sperm concentration ($r = 0.66$, $n = 10$, $P = 0.04$) and we obtained more total sperm from them ($r = 0.65$, $n = 10$, $P = 0.04$). Moreover, Captive sparrows with more black on their beaks had greater CTM ($r = 0.61$, $n = 10$, $P = 0.06$).

On 14 July, a significantly larger proportion of the beaks of Wild sparrows was black (1.00 ± 0.0, n = 9) compared to the proportion of black on the beaks of Captive sparrows (0.74 ± 0.26, n = 10, range 0.28 – 1.00) (Mann-Whitney U = 10.5, P = 0.007).

The mean CTM of Captive sparrows (0.079 ± 0.128 g; range, 0.003 – 0.405 g) was significantly less than that of Wild sparrows (0.692 ± 0.326 g; range, 0.426 – 1.402 g) ($t_{15} = 5.44$, $P < 0.001$). Seminal glomera were easily found and resected in the Wild sparrows but were difficult to find and resect from Captive sparrows and so were not

weighed. Examination under the microscope of the left testes of three Captive (Captive No. 14 = 0.004 g, Captive No. 15 = 0.089 g, Captive No. 22 = 0.004 g) and three Wild males (Wild No. 35 = 0.58 g, Wild No. 36 = 0.25 g, Wild No. 39 = 0.31 g) revealed sperm in the testes of Wild but not Captive sparrows. The left seminal glomera of the same three Wild sparrows contained sperm cells.

We could not statistically examine the relationship between CTM and sperm production in Captive sparrows because we obtained semen containing sperm from only 2/17 males on 12 July. In contrast, CTM was not associated with semen volume ($r = 0.69$, $n = 7$, $P = 0.09$), sperm concentration ($r = 0.06$, $n = 7$, $P = 0.91$), or the total amount of sperm obtained ($r = 0.51$, $n = 7$, $P = 0.25$) from the seven euthanized Wild sparrows.

The Captive sparrow caged with a female from 31 May – 14 July stopped producing semen by 30 June. We could not obtain semen samples from him on 15 and 18 June and his semen samples on 22 June (volume = 4 µL) and 26 June (volume = 3 µL) lacked sperm cells. When the male was euthanized on 15 July his (a) beak was 28 percent black, (b) CTM was 0.003 g, and (c) left testis was devoid of sperm cells.

Captive and Wild sparrows were morphologically similar, but differed in mass on the days they were captured (Table 1).

Table 1
Comparison between the morphological characteristics of euthanized Captive (n = 10) and Wild sparrows (n = 7). Means ± SD are illustrated. U = Mann-Whitney U test.

Charakter	Captive Sparrow	Wild Sparrows	U	P
Bib area (mm^2)	392.72 ± 91.73	399.16 ± 80.87	32	0.81
Bill (mm)	14.05 ± 2.90	12.24 ± 0.56	18	0.11
Right tarsus (mm)	17.86 ± 2.74	19.07 ± 0.86	27.5	0.48
Right wing chord (mm)	75.60 ± 1.58	73.71 ± 1.70	16	0.07
Mass at capture (g)	25.79 ± 1.92	22.76 ± 2.04	7	0.01

DISCUSSION

Our results indicate that being held individually in captivity with repeated semen sampling was associated with decreased sperm production, testicular atrophy, and beak color change in male House Sparrows. This was surprising. First, Captive sparrows should have remained in breeding condition throughout the sampling period from 15 June – 12 July which coincided with the middle of the House Sparrow breeding season in west Michigan (Berger 1957). Second, House Sparrows have been bred, albeit with difficulty, in captivity (Baker 1995, Mitchell and Hayes 1973, Moreno-Rueda and Soler 2002) with greater breeding success in groups of House Sparrows caged together than in isolated pairs (Anderson 2006). Moreover, Hegner and Wingfield (1984) suggested that social facilitation may be essential for gonadal development in male House Sparrows

because they observed greater testicular recrudescence in males housed in flocks than in males housed in pairs. Thus, the decline in sperm production and testicular atrophy in Captive males may be associated with the lack of social interactions between them.

We think that T levels declined in Captive sparrows for several reasons. First, the decrease in sperm production in Captive sparrows suggests a decline in T because spermatogenesis is stimulated by T (Goes and Dolder 2002, Johnson 1986, Wingfield and Farner 1993). Second, the testicular atrophy of Captive sparrows suggests a decline in T because the size of House Sparrow testes, the primary source of T (Fevold and Eik-Nes 1962), is positively correlated with circulating T levels (Hegner and Wingfield 1986a, Hegner and Wingfield 1986b) as it is in other bird species (Denk and Kempenaers 2006, Garamszegi et al. 2005). House Sparrow testes remain at or near their maximum size during the breeding season (Hegner and Wingfield 1986b) when higher T levels are associated with competition for nest sites and mates (Hegner and Wingfield 1986a). Photoperiod is the most important external stimulus affecting testes development in House Sparrows (Kendeigh 1941, Riley 1937), although social interactions are also important (Hegner and Wingfield 1984). Captive sparrows were exposed to natural photoperiods but were kept from direct social interactions because each was caged alone. The testes and seminal glomera of Captive sparrows were significantly smaller than those of Wild sparrows also implying a decline in T in Captive sparrows. The differences in CTM between Captive and Wild sparrows paralleled those found between the testes of male House Sparrows during the nonbreeding and breeding seasons, respectively (Keck 1934). Moreover, the testes and seminal glomera of Wild sparrows contained sperm whereas the testes of the Captive sparrows we examined did not. This is additional evidence that T levels declined in Captive sparrows. Last, the decrease in black on the beaks of Captive sparrows over the course of the sampling period suggests a decline in T because beak color is correlated with T level (Haase 1975, Keck 1933, Keck 1934, Lofts et al. 1973) and is a reliable indicator of testicular activity (Kendeigh 1941). Beaks are black during the breeding season when T levels are high and become horn-colored during the non-breeding season when T levels are low (Hegner and Wingfield 1986a, b). Fevold and Eik-Nes (1962) found that a CTM of 0.005-0.008 g is necessary for deposition of melanin in the beak. Only one Captive sparrow had a CMT (0.003 g) below this threshold, and his beak had the least amount of black (28 percent). House Sparrows typically have a long breeding season that extends from mid-March to mid-September and males have black beaks during this period (Lowther and Cink 1992). The change in beak color of Captive sparrows and concomitant decrease in CTM are consistent with the hypothesis that T production declined during the time that sparrows were held captive.

Moreover, the testes of Captive sparrows decreased in size unusually early in the season (Berger 1957, Lowther and Cink 1992, Anderson 2006). The synchronous regression of gonad size has been observed in other captive non-domesticated wild birds

but usually in late summer (Wingfield and Farner 1979). Thus the timing of the nearly synchronous regression of testes size, as indicated by changes in beak color, in Captive sparrows was surprising because it occurred in the middle of the breeding season.

In House Sparrows, the stress of capture, confinement in small cages (Lynn and Porter, 2008), and handling (Lynn and Porter 2008, Rich and Romero 2001, Romero and Romero 2002) are all associated with the secretion of the steroid hormone corticosterone (CORT). Prolonged elevation of CORT may suppress reproduction in birds (Deviche 1983, Silverin 1986). Furthermore, the stress of captivity may be exacerbated by small cage size (Cancione et al. 2002, Lynn and Porter 2008). Indeed, short-term confinement (≤ 90 min) in wire cages smaller (18 cm x 18 cm x 18 cm) than those used in our study (see above) resulted in a marked CORT response in House Sparrows (Lynn and Porter 2008). While acute stress can result in short-term elevations of CORT (Wingfield and Kitaysky 2002), chronic stress typically results in prolonged elevation of CORT and is associated with significant changes in behavior and physiology (Wingfield et al. 1998) including gonadal atrophy (Wingfield 1994). The changes in behavior and physiology are likely due to the proposed reciprocal relationship between CORT and T (Greenberg and Wingfield 1987, Wingfield and Kitaysky 2002). Thus, the decrease in sperm production, testicular atrophy, and the change in beak color in Captive sparrows may have resulted from prolonged elevated CORT levels that were due the stress of captivity and frequent handling. This hypothesis may be tested with hormone assays.

Captive breeding of House Sparrows has been most successful when sparrows have been housed in cages that allowed each individual from 0.4 m^3 (Moreno-Rueda and Soler 2002) – 5.5 m^3 (Baker 1995) of space. Our birds, housed alone had 0.03 m^3 of space. Studies of captive breeding of House Sparrows report greater success in groups caged together than in isolated pairs, but captive breeding is difficult and fledging success is typically lower than that of free-living sparrows in the same area (Anderson 2006). The greater reproductive success found in groups of sparrows housed together implies that male House Sparrows may require physical contact to maintain sperm production. Our results imply that just seeing or hearing other House Sparrows in close proximity and the presence of nest boxes and nest material were not sufficient for Captive sparrows to maintain sperm production and black beak color, and by implication breeding season T levels. This contrasts with other studies (Dufty and Wingfield 1986, Feder et al. 1977, Pinxten et al. 2003) that demonstrated that social cues and the presence of nest sites and nesting material help stimulate gonadal activity in male birds.

Several aspects of the reproductive physiology of male birds allow us to exclude several alternative explanations for our observations. First, it is unlikely that the decline in sperm production in Captive sparrows was caused by the type of food we provided them. Captive sparrows were fed a commercial product specifically designed for birds that we supplemented with another product designed to enhance breeding by caged birds. Furthermore, the Captive sparrows were fed ad libitum, so it is unlikely that sperm

production declined because of a lack of food (Kendeigh 1941). In addition, experiments by (a) Meijer and Schwabl (1989) showed that access to food by captive male Kestrels (*Falco tinnunculus*) had little effect on the levels of T and luteinizing hormone the two hormones that most directly affect testes development and spermatogenesis and (b) Meijer and Langer (1995) showed that the amount of food consumed had little effect on sperm production in captive European Starlings so long as the amount of food consumed exceeded starvation levels.

Second, it is unlikely that the lack of physical activity negatively affected sperm production in Captive sparrows. Activity patterns had little effect on sperm production in the captive House Sparrows studied by Riley (1937) and Kendeigh (1941).

Third, repeated sampling per se is unlikely to have negatively affected sperm production and testes size in Captive sparrows. Sperm production is not affected by the frequency of ejaculation (Amann 1981). We sampled Captive sparrows every third day which should have allowed them plenty of time to "recharge" their sperm supplies (Bird and Laguë 1976) especially because free-ranging House Sparrows copulate frequently (Birkhead et al. 1994, Lowther and Cink 1992) and have relatively large sperm reserves (Birkhead et al. 1994). However, we found that sperm concentrations declined during the period Captive sparrows were held captive and that the testes of all the three Captive males that were histologically examined lacked sperm.

Fourth, we are unaware of any reports of testicular atrophy resulting from frequent ejaculation or sampling. However, handling Captive sparrows every third day may have resulted in chronically elevated CORT levels (Lynn and Porter 2008, Romero and Romero 2002) which ultimately resulted in a decline in T production and testicular atrophy. This possibility can be directly tested with hormone assays. Experiments in 2006 during which we attempted to obtain semen samples from captive male House Sparrows only once a week (Lombardo and Thorpe – unpublished) produced the same results as described here suggesting that male House Sparrows may especially sensitive to the stresses of captivity and handling (contra American Kestrels, Bird and Laguë 1976). Housing conditions had no effect on follicular development in female House Sparrows (Hegner and Wingfield 1984).

Last, we do not think that intrinsic differences between subpopulations (cf. Lambrechts et al. 1999) of House Sparrows in Ottawa County, MI were responsible for the differences between Captive and Wild sparrows in sperm production and testes size. All sparrows were captured within 16 km of the GVSU campus, so we think it was unlikely that some males stopped producing sperm while other males nearby continued to produce sperm.

In conclusion, our results show that being housed alone in captivity and repeatedly handled negatively affected sperm production, testes size, and beak color in male House Sparrows. We concur with Anderson (2006) that House Sparrows do not acclimate well to captivity and hypothesize that the difficulty in breeding them in captivity may stem

from the effects of captivity on CORT levels. This hypothesis can be directly tested with hormone assays. The question of why House Sparrows seem to be especially susceptible to the stresses of captivity and handling remains unanswered.

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REFERENCES

Amann, R. P. 1981 – A critical review of methods for evaluation of spermatogenesis from seminal characteristics – J. Androl., 2:37-58.

Anderson, T. R. 2006 – Biology of the ubiquitous House Sparrow: from genes to populations – Oxford University Press, New York, NY.

Baker, M. 1995 – Environmental component of latitudinal clutch size variation in House Sparrows (*Passer domesticus*) – Auk, 112:249-253.

Bakst, M. R., Cecil, H. C. – 1977. Techniques for semen evaluation, semen storage, and fertility demonstration – The Poultry Science Association, Inc., Savoy, IL.

Berger, A. J. 1957 – Nesting behavior of the House Sparrow – Jack Pine Warbler, 35:86-92.

Bird, D. M., Laguë, P. C. 1976 – Management practices for captive kestrels used as semen donors for artificial insemination – Raptor Res., 10:92-96.

Birkhead, T. R., Veiga, J. P., Møller, A. P. 1994 – Male sperm reserves and copulation behaviour in the House Sparrow, *Passer domesticus* – Proc. R. Soc. Lond. B, 256:247-251.

Cancione, V., Hayden, T. J., Rowe, K., Goymann, W. 2002 – The stress response of European Stonechats depends on the type of stressor – Behav., 139:1303-1311.

Denk, A. G., Kempenaers, B. 2006 – Testosterone and testes size in Mallards (*Anas platyrhynchos*) – J. Ornithol., 147:436-440.

Deviche, P. 1983 – Interactions between adrenal function and reproduction in male birds. In: Avian endocrinology: environmental and ecological perspectives. Eds. Mikami, S., Ichii, S., Wada, M – Springer, Berlin: 243-254.

Dufty, Jr., A. M., Wingfield, J. C. 1986 – The influence of social cues on the reproductive endocrinology of male Brown-headed Cowbirds: field and laboratory studies – Horm. Behav., 20:222-234.

Feder, H. H., Storey, A., Goodwin, D., Reboulleau, C., Silver, R. 1977 – Testosterone and 5a-dihydrotestosterone levels in peripheral plasma of male and female Ring Doves (*Streptopelia risoria)* during the reproductive cycle – Biol. Reprod., 16:666-677.

Fevold, H. R., Eik-Nes, K. B. 1962 – Progesterone metabolism by testicular tissue of the English Sparrow (*Passer domesticus*) during the annual reproductive cycle – Gen. Comp. Endocrinol., 26:248-252.

Garamszegi, L. Z., Eens, M., Hurtrez-Boussès, S., Møller, A. P. 2005 – Testosterone, testes size, and mating success in birds: a comparative study – Horm. Behav., 47:389-409.
Gee, G. F., Bertschinger, H., Donoghue, A. M., Blanco, J., Soley, J. 2004 – Reproduction in non-domestic birds: physiology, semen collection, artificial insemination and cryopreservation – Avian Poult. Rev., 15:47-101.
Gee, G. F., Sexton, T. J. 1983 – Seaside Sparrow sperm preservation: The Seaside Sparrow, its biology and management 1-2 October 1981:163-165.
Gee, G. F., Temple, S. A. 1978 – Artificial insemination for breeding non-domestic birds – Symp. Zool. Soc. Lond., 43:51-72.
Goes, R. M., Dolder, H. 2002 – Cytological steps during spermiogenesis in the House Sparrow (*Passer domesticus,* Linnaeus) – Tissue & Cell, 34:273-282.
Greenberg, N., Wingfield, J. C. 1987 – Stress and reproduction: reciprocal relationships. In: Reproductive endocrinology of fishes, amphibians and reptiles. Eds. D. O. Norris, R. E. Jones, Wiley, New York: 389-426.
Haase, E. 1975 – The effects of testosterone propionate on secondary sexual characters and testes of House Sparrows, *Passer domesticus* – Gen. Comp. Endocrinol., 26:248-252.
Hegner, R. E., Wingfield, J. 1984 – Social facilitation of gonadal recrudescence -J. Steroid Biochem., 20:1549.
Hegner, R. E., Wingfield, J. C. 1986a – Behavioral and endocrine correlates of multiple brooding in the semicolonial House Sparrow Passer domesticus II. Females – Horm. Behav., 20:313-326.
Hegner, R. E., Wingfield, J. C. 1986b – Gonadal development during autumn and winter in House Sparrows – Condor, 88:269-278.
Johnson, A. L. 1986 – Reproduction in the male. In: Avian physiology, Ed. P. D. Sturkie – Springer-Verlag, New York, NY: 432-451.
Keck, W. N. 1933 – Control of bill color of the male English Sparrow by injections of male hormone – Proc. Soc. Exp. Biol. Med., 30:1140-1141.
Keck, W. N. 1934 – The control of secondary sex characteristics in the English sparrow, *Passer domesticus* Linnaeus – J. Exper. Zool., 190:137-153.
Kendeigh, S. C. 1941 – Length of day and energy requirements for gonad development and egg-laying birds – Ecology, 22:237-248.
King, A. S. 1981. Cloaca. In: Form and function in birds, Eds. A. S. King, J. McLelland – Academic Press, New York: 107-148.
Lambrechts, M. M., Perret, P., Maistre, M., Blondel, J. 1999 – Do experiments with captive non-domesticated animals make sense without population field studies? A case study with Blue Tits' breeding time – Proc. R. Soc. Lond. B, 266:1311-1315.
Lofts, B., Murton, R. K., Thearle, R. J. P. 1973 – The effects of testosterone propionate and gonadotropins of the bill pigmentation and testes of the House Sparrow (*Passer domesticus*) – Gen. Comp. Endocrinol., 21:202-209.
Lowther, P. E., Cink, C. L. 1992 – House Sparrow. In: The birds of North America, Eds. A. Poole, P. Stettenheim, F. Gill – The Academy of Natural Sciences, Philadelphia, PA, The American Ornithologists' Union., Washington, D. C.
Lynn, S. E., Porter, A. J. 2008 – Trapping initiates stress response in breeding and non-breeding House Sparrows *Passer domesticus*: implications for using unmonitored traps in field studies – J. Avian Biol., 39:87-94.
Meijer, T., Langer, U. 1995 – Food availability and egg-laying of captive European Starlings – Condor, 97:718-728.

Meijer, T., Schwabl, H. 1989 – Hormonal patterns in breeding and nonbreeding Kestrels, *Falco tinnunculus*: field and laboratory studies – Gen. Comp. Endocrinol., 74:148-160.
Mitchell, C. J., Hayes, R. O. 1973 – Breeding House Sparrows, *Passer domesticus* in captivity – Ornithol. Monog., 14:39-48.
Moreno-Rueda, G., Soler, M. 2002 – Cría en cautividad del gorrión común *Passer domesticus* – Ardeola, 49:11-17.
Pinxten, R., deRidder, E., Eens, M. 2003 – Female presence affects male behavior and testosterone levels in the European Starling (*Sturnus vulgaris*) – Horm. Behav., 44:103-109.
Rich, E. L., Romero, L. M. 2001 – Daily and photoperiod variations of basal and stress-induced corticosterone concentrations in House Sparrows (*Passer domesticus*) – J. Comp. Physiol. B, 171:543-547.
Riley, G. M. 1937 – Experimental studies of spermatogenesis in the House Sparrow, *Passer domesticus* (Linnaeus) – Anat. Rec., 67:327-351.
Romero, L. M., Romero, R. C. 2002 – Corticosterone responses in wild birds: the importance of rapid initial sampling – Condor, 104:129-135.
Samour, J. H., Smith, C. A., Moore, H. D., Markham, J. A. 1986 – Semen collection and spermatozoa characteristics in Budgerigars – Vet. Rec., 118:397-399.
Silverin, B. 1986 – Corticosterone binding proteins and behavioral effects of high plasma levels of corticosterone during the breeding period in Pied Flycatchers – Gen. Comp. Endocrinol., 64:67-74.
Snyder, N. F. R., Derrickson, S. R., Beissinger, S. R., Wiley, J. W., Smith, T. B., Toone, W. B., Miller, B. 1996 – Limitations of captive breeding in endangered species – Con. Biol., 10:338-348.
SPSS. 2002 – SPSS for windows, Version 10.0 – SPSS, Chicago, IL.
Wingfield, J. C. 1994 – Modulation of the adrenocortical response to stress in birds. In: Perspectives in comparative endocrinology, Eds. K. G. Davey, R. E. Peter, S. S. Tobe – National Research Council, Ottawa, Canada: 520-528.
Wingfield, J. C., Farner, D. S. 1979 – Some endocrine correlates of renesting after loss of clutch or brood in the White-crowned Sparrow, *Zonotrichia leucophrys gambeii* – Gen. Comp. Endocrinol., 38:322-331.
Wingfield, J. C., Farner, D. S. 1993 – Endocrinology of reproduction in wild species. In: Avian biology, Eds. D. S. Farner, J. R. King, K. C. Parkes – Academic Press, New York, NY: 163-327.
Wingfield, J. C., Kitaysky, A. S. 2002 – Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? – Integ. Comp. Biol., 42:600-609.
Wingfield, J. C., Maney, D. L., Breuner, E. W., Jacobs, J. D., Lynn, S., Ramenofsky, M., Richardson, R. D. 1998 – Ecological bases of hormone-behavior interactions: the "emergency life history stage" – Am. Zool., 38:191-206.
Wolfson, A. 1954 – Notes on the cloacal protuberance, seminal vesicles, and a possible copulatory organ in male passerine birds – Bull. Chicago Acad. Sci., 10:1-23.