

Seasonal Fluctuation in Concentration of Progesterone and Testosterone in Three Avian Species - *Acridotheres ginginianus* (Sturnidae), *Sturnus pagodarum* (Sturnidae) and *Turdoides striatus* (Muscicapidae), with Diverse Breeding Strategies

*Bharucha Bhavna**, *Surendran Sapna*, *Padate Geeta*

Division of Avian Biology, Department of Zoology, Faculty of Science,
The M. S. University of Baroda, Vadodara, Gujarat, India

Abstract: Stereotyped reproductive behavioural activities of birds are known to reflect the varying intensities of male and female sex hormones, i.e. testosterone and progesterone in both sexes. The elevated levels of testosterone influence courtship and nest-building activities both in male and female birds. On the other hand, progesterone is known to occur in male birds at basal levels all throughout the reproductive cycle or at slightly elevated levels during breeding season. The present study reports the testosterone and progesterone levels in plasma over the reproductive cycles of male and female individuals of three species of birds, two seasonally reproducing species, Bank Myna (*Acridotheres ginginianus*), a colonial hole nester, Brahminy Myna (*Sturnus pagodarum*), an solitary hole nester (Family Sturnidae) and a social, cooperative breeder, Jungle Babbler (*Turdoides striatus*) (Family Muscicapidae). The males of both species of Mynas have highest testosterone levels during breeding phase and comparatively low titers during pre-breeding and post breeding and lowest in non-breeding phase. Females also showed higher testosterone level indicating their involvement in courtship and nest defense activities. However the titers were low in the colonial nester compared to the solitary nester. In the Jungle Babbler, non-significant difference in the plasma testosterone levels were observed in the breeding males and females as well as the helper females suggesting that the various reproductive activities are combined flock actions. Progesterone, the major female sex steroid, was present at a basal level all throughout the cycle in male mynas and at higher level during breeding season in the female Mynas. Similarly increased levels of progesterone in the male Jungle Babbler can be related to social breeding habits wherein males are also involved in incubation while in females variation is synchronous with the egg-laying activity. Helper females with intermediate progesterone levels indicate their allo-parental behavior.

Key words: testosterone, progesterone, social breeder, solitary breeder, colonial breeder

Introduction

One of the most fascinating aspects in the life of birds is their breeding phase, which is intimately tied to the distribution and abundance of food resources, nesting material and nesting sites in their environment. However, these are not evenly distributed in space and time, and these are thought to be the significant factors affecting the evolution of unusual nesting strategies. The three fundamental types of

*Corresponding author: cyprea_bb@yahoo.com

breeding patterns observed amongst the birds are solitary nesters, colonial nesters and social/ co-operative breeders.

Among the passerines, most species are solitary nesters and few are colonial or social/co-operative breeders. A variety of reasons for the evolution of these varied nesting strategies have been proposed. For smaller species, small, cryptic nests are favoured. Solitary hole nesters (eg Brahminy myna, *Sturnus pagodarum*) reuse cavities made by other birds or are natural. During breeding season only the breeding pair is responsible for all the chores of reproduction. This type of solitary nesting has its own advantages and disadvantages. But larger the bird, greater problem of concealment of the nests, but have better prospects of active nest defense when nesting colonially. Synchronization in the timing of breeding is a common feature of the reproductive biology of many colonial nesting birds. A high degree of gregariousness and common responsiveness to the same environmental factors trigger breeding in colonial nesters. The Bank myna (*Acridotheres ginginianus*) is a gregarious bird that breeds in dense colonies ranging from 10 to 300 pairs. The birds dig their nests 0.5 to 1.3 m deep into vertical embankments along rivers.

Co-operative breeding differs from other breeding patterns seen in birds, in that they have group members who share most of the reproductive activities with others and these adults, termed 'helpers' remain reproductively dormant and help raise the young of breeding pairs to whom they are often, but not always related (DAWSON, MANNAN 1991, WALTERS 1990). In these systems, the evolution of cooperative breeding involves two distinct phenomena: the young remain on their natal territory, rather than dispersing and the retained young express allo-parental behavior (BROWN 1987, EMLÉN 1982a, 1982b). Jungle Babbler (*Turdoides striatus*) occurs in groups that consist of a single breeding female, a dominant male breeder, and up to 3 subordinate, non breeding helpers, most of which are female. Surprisingly, very few quantitative studies have dealt with breeding physiology and reproductive endocrinology of these diverse nesters. Thus, as a part of the present study, was to obtain basic physiological information of breeding biology of these three birds.

Reproductive behaviour in both males and females in all avian species is strongly correlated with elevated plasma Testosterone, Progesterone, Estrogen titers in synchrony with prolactin concentration. Endocrine studies have greatly expanded our understanding of hormonal interactions with social and ecological signals that affect the timing, sequence and frequency of the reproductive activities (MORTON *et al.* 1990). Hormones are highly potent biomolecules that exhibit tissue specific activity and regulation. Thus, a valuable source of information regarding the relationship between hormones and sexual behaviour lies in understanding the development of sex-specific and species-specific differences and similarities (BALTHAZARD *et al.* 1996). It is well known that in males testosterone is required for normal sexual behaviour. In birds, testosterone induces courtship, establishment of territory, nest-building and other related activities (DAWSON 1983, DELVILLIE *et al.* 1984, HAU *et al.* 2000, SOMA *et al.* 2000, RIETERS *et al.* 2002). Secondary sexual characteristics that may differentiate the male from female, like size of the comb, plumage and bill colour, structure of feathers, vocalizations and behaviour especially during the breeding season are all under the influence of testosterone (JOHNSON 1986b; WELTY AND BAPTISTA 1990). In females, progesterone is known to contribute to changes in the ovarian cycle. Decreased levels of progesterone are known to cause follicular atresia, inhibition of ovulation, egg formation and also induce molting in birds (JOHNSON 1986a). Evidently, progesterone has an indispensable role in reproduction. STUAB, MADELEINE DE BEER (1997) have reviewed the role of androgens in female vertebrates and an investigation on its role has been suggested as a promising approach to learn about androgen functions in females. Thus the purpose of the present study was to investigate the basic physiological/endocrinological information on breeding of three avian species viz. Bank myna (colonial nester); Brahminy myna (solitary hole nester) and Jungle babbler (social/co-operative breeder) with different breeding strategies. Measurement of circulating hormones, testosterone (T) and progesterone (P) as well as body weights was measured during different phases of their reproductive cycle.

Materials and Methods

The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India and approved by the Animal Ethical Committee of Department of Zoology, The MS University of Baroda, Vadodara.

The model bird species, Bank Myna (*Acridotheres ginginianus*), Brahminy Myna (*Sturnus pagodarum*) and Jungle Babbler (*Turdoides striatus*), are common birds of the Indian subcontinent. Bank Myna is a colonial hole nester and Brahminy Myna is an individual hole nester, whereas Jungle Babbler is a social breeder where individuals called helpers assist in nesting activities (ALI 1993). Depending on the field observations, the birds were obtained from a local bird supplier during different phases of their breeding cycle as well as in the non-breeding phase and were kept in the Zoology Department aviary. Food and water were supplied *ad libitum*. Ten birds per phase (pre-breeding, breeding, post-breeding and non-breeding) were used in the two sturnid species, while for Jungle babbler ten birds each for breeding and non-breeding males and females and helpers respectively were used for hormonal study. The blood samples were collected early in the morning between 05:30 h and 7:00 h IST. Before sacrificing, blood was collected in the heparinised test tubes from the ventricles of the anesthetized birds and later centrifuged for 60 min at 5000 rpm. After centrifugation plasma was collected in Eppendorf tubes and stored at -20 °C. The separated plasma fraction (10 µl) was used for quantitative measurements of progesterone and testosterone (by EIAgen kit, Biochem; Italy) respectively in all the three avian species during different phases of breeding.

EIAgen Testosterone/Progesterone kit

To evaluate testosterone/progesterone content in blood plasma, a microplate solid phase enzyme immunoassay kit was used. The EIAgen testosterone/ progesterone kit contains: a testosterone/ progesterone microplate, testosterone/ progesterone calibrators, testosterone/ progesterone conjugates, washing solution, TMB H₂O₂ HS, stop solution (H₂SO₄). The solid phase enzyme immunoassay for testosterone/ progesterone is a competitive type immunoassay

wherein HRP labeled testosterone/ progesterone competes with the testosterone/ progesterone present in the sample (10 µl) for a fixed and limited number of antibody sites immobilised on the wells of the microstrips. Once the competitive immunoassay reaction has occurred, the wells are washed and the HRP-testosterone/ progesterone fraction bound to the antibody in the solid phase is measured by adding the chromogen/ substrate solution which is converted to a blue compound. After 15 min of incubation, the enzyme reaction is stopped with H₂SO₄, which also changes the solution to yellow. The absorbance of the solution is measured photometrically at 450 nm and is inversely related to the concentration of the testosterone/ progesterone present in the sample (10 µl). Calculations of the testosterone/ progesterone content in the sample are made by reference to a calibration curve.

Calculations of results for testosterone/ progesterone

To calculate the mean absorbance of calibrators and samples (A), the absorbance of the chromogen blank (Ac) is subtracted from the absorbance of all the samples. This is considered as the corrected value. Corrected values of the sample are divided by the corrected absorbance of the zero calibrator (Ao) and multiplied by 100 ($A - Ac / Ao - Ac \times 100$). The respective testosterone/ progesterone values are plotted on the logit log or semi log graph paper and the concentration of testosterone/ progesterone in the samples are determined by the interpolation from the calibration curves.

Statistics

Statistical evaluation of the data was done by Student's *t*- test (non-parametric test), one way ANOVA followed by Bonferroni post test and results are expressed as mean ± S.E. (P<0.0001: ***/◆◆◆, P<0.001: **/ ◆◆, P<0.01: */◆, P>0.05: non-significant), using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego California, USA.

The reason for the sacrifice of these birds was to understand the biochemical physiology in depth and to explore the feasibility of incorporating these birds in the agricultural pest management (as bio-control agents). The study carried out was funded

by ICAR (Indian Council of Agricultural Research) due to the importance of the birds in IPM (Integrated Pest Management) approach. The objective of the project was to assess the relationship of carbohydrate, protein and lipid metabolism along with the histology, histochemistry and reproductive/breeding physiology including hormonal interaction during different seasons.

Results

Testosterone levels

Mynas (Fig. 1, 2)

In Bank Myna males, higher levels, i.e. 0.65 ± 0.03 ng testosterone/ml blood plasma was present during the pre-breeding season, which increased significantly to 1.5 ± 0.06 ng/ml of blood plasma during the breeding season. Thereafter a steady decrease to 0.55 ± 0.03 ng/ml in the post-breeding phase and to 0.33 ± 0.05 ng/ml {F = 133.1, t = (PrB Vs B= 13.52, P<0.001, PrB Vs PoB= 1.591, ns, PrB Vs NB= 5.092, P<0.001, B Vs PoB= 15.12, P<0.001, B Vs NB = 18.62, P<0.001, PoB Vs NB= 3.500) ns} during the non-breeding phase was noted. In the other species, Brahminy Myna also, similar pattern of variation was seen except that during the breeding season plasma testosterone levels were comparatively lower and during pre-breeding and post-breeding seasons they were higher than those noted for Bank Myna. In this species, plasma testosterone levels were 0.93 ± 0.05 ng/ml during the pre-breeding season, which rose to 2.08 ± 0.08 ng/ml during the breeding season and decreased gradually to 0.98 ± 0.06 ng/ml during the post-breeding season and further decreased to 0.35 ± 0.02 ng/ml {F = 161.8, t = (PrB Vs B= 14.32, P<0.001, PrB Vs PoB= 0.62, ns, PrB Vs NB= 7.222, P<0.001, B Vs PoB= 13.70, P<0.001, B Vs NB = 21.54, P<0.001, PoB Vs NB= 7.84, P<0.001)} during the non-breeding season. The increase during the breeding season was of lower amplitude in the later species.

Progesterone levels

Mynas (Fig. 3, 4)

Progesterone, one of the female sex hormones was high only during breeding phase in females of both species of Mynas, which was 1 ng/ml of blood plasma. During the other phases of reproduction, i.e. pre-

breeding, post-breeding and non-breeding phases, it was almost maintained at 0.1 ng/ml of blood plasma in both Mynas. No prominent species-specific difference was noted in the female birds. For Bank myna female the values being {F = 900, t = (PrB Vs B= 42.43, P<0.001, PrB Vs PoB= 0.00, ns, PrB Vs NB= 0.00, ns, B Vs PoB= 42.43, P<0.001, B Vs NB = 42.43, P<0.001, PoB Vs NB= 0.00) ns}, while that for Brahminy myna females was {F = 747.4, t = (PrB Vs B= 38.38, P<0.001, PrB Vs PoB= 0.4264, ns, PrB Vs NB= 0.4264, ns, B Vs PoB= 38.80, P<0.001, B Vs NB = 38.80, P<0.001, PoB Vs NB=0.00) ns}. In the male birds, a basal level of plasma progesterone was noted at 0.1 ng/ml all through out the reproductive cycle in both species. In Brahminy myna males the values were {F = 0.666, t = (PrB Vs B= 1, PrB Vs PoB= 0.00, PrB Vs NB= 1, B Vs PoB= 1, B Vs NB = 0, PoB Vs NB= 1)}. In females of both species of sturnids the values were non-significant during all the stages of breeding cycle.

Jungle Babbler (Fig. 5)

The testosterone levels in the breeding and the non-breeding males were 0.76 ± 0.17 ng/ml of blood plasma and 0.21 ± 0.040 ng/ml (F = 18.06, t = 7.71) respectively. The mean testosterone levels in the female birds of both the species of Mynas during the pre-breeding season were 0.23 ng/ml of blood plasma. These levels rose to 1.5 ng/ml in both Mynas during the breeding season. When both species entered the post-breeding season, a sharp decrease to 0.3 ng/ml of blood plasma was noted which was maintained till the non-breeding season ended. For Bank myna female the values being {F = 179.1, t = (PrB Vs B= 19.80, P<0.001, PrB Vs PoB= 1.027, ns, PrB Vs NB= 1.760, ns, B Vs PoB= 18.77, P<0.001, B Vs NB = 18.04, P<0.001, PoB Vs NB= 0.733) ns}, while that for Brahminy myna females was {F = 241.2, t = (PrB Vs B= 23.19, P<0.001, PrB Vs PoB= 1.278, ns, PrB Vs NB= 2.73, ns, B Vs PoB= 21.91, P<0.001, B Vs NB = 20.45, P<0.001, PoB Vs NB=1.461) ns}. The testosterone levels in the breeding and the non-breeding females of Jungle Babbler were 0.65 ± 0.050 ng/ml of blood plasma and 0.18 ± 0.049 ng/ml respectively; while in the helper females it was 0.63 ± 0.033 ng/ml of blood plasma {F = 36.53, t = (B Vs NB= 7.558, P<0.001, B Vs H= 0.32, ns, NB Vs H= 7.236) P<0.001}.

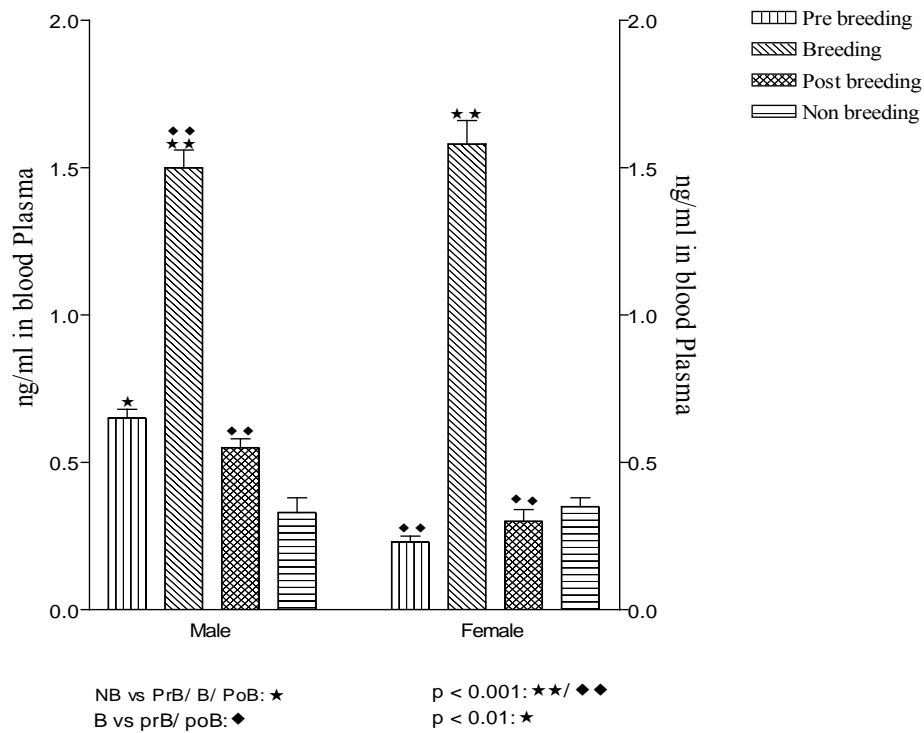


Fig. 1. Testosterone levels during breeding and non breeding season in male and female Bank Myna (*Acridotheres ginginianus*).

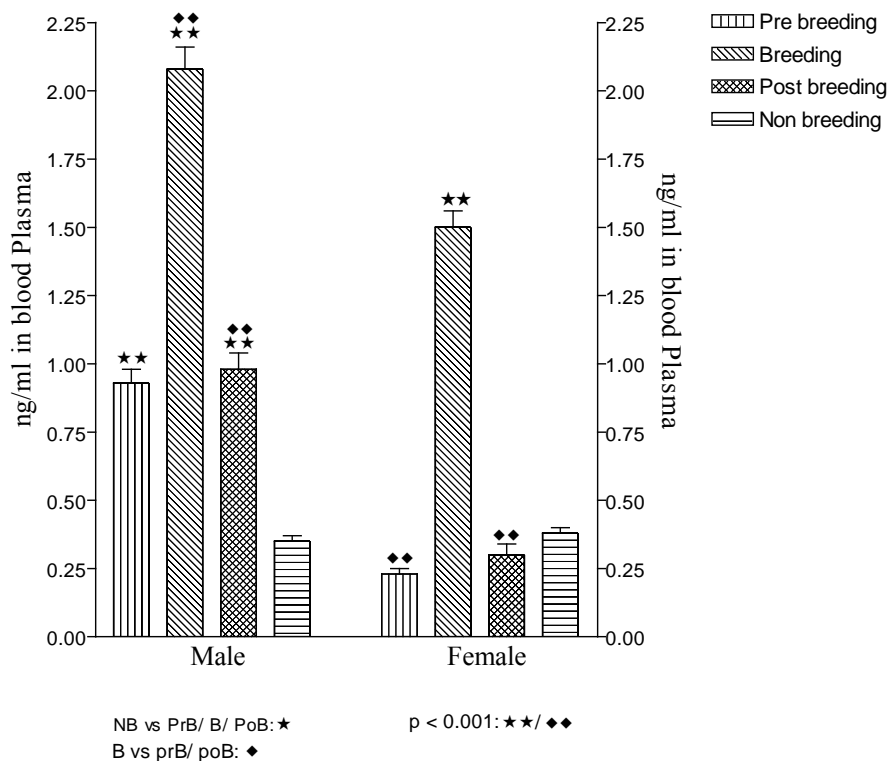


Fig. 2. Testosterone levels during breeding and non breeding season in male and female Bhraminy Myna (*Sturnus pagodarum*).

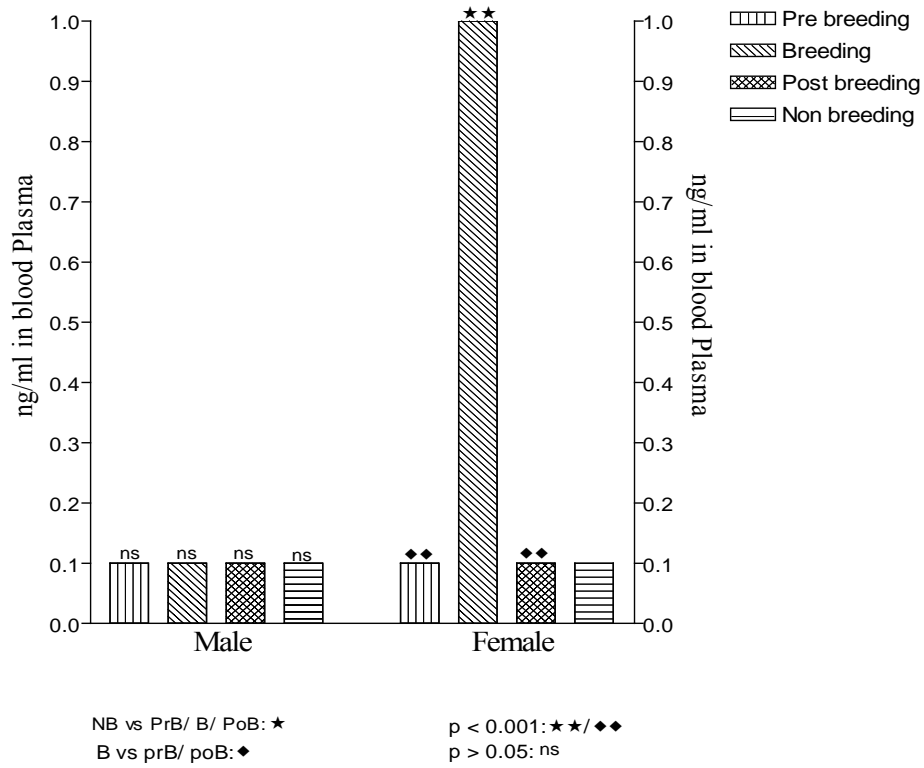


Fig. 3. Progesterone levels during breeding and non breeding season in male and female Bank Myna (*Acridotheres ginginianus*).

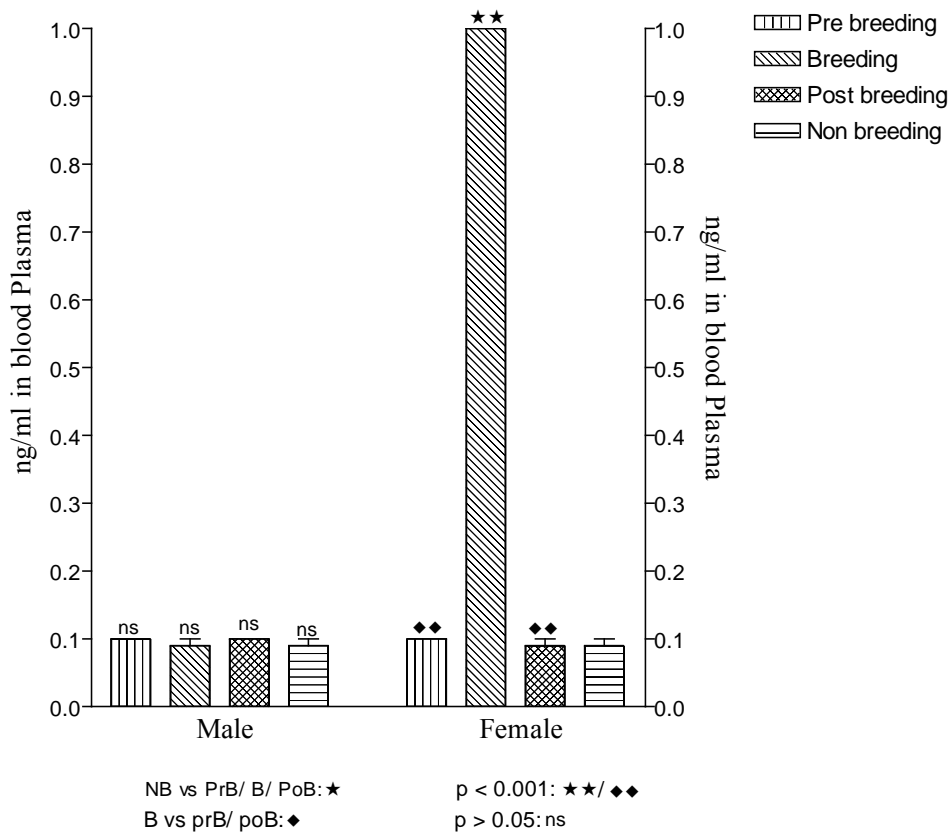


Fig. 4. Progesterone levels during breeding and non breeding season in male and female Bhraminny Myna (*Sturnus pagodarum*).

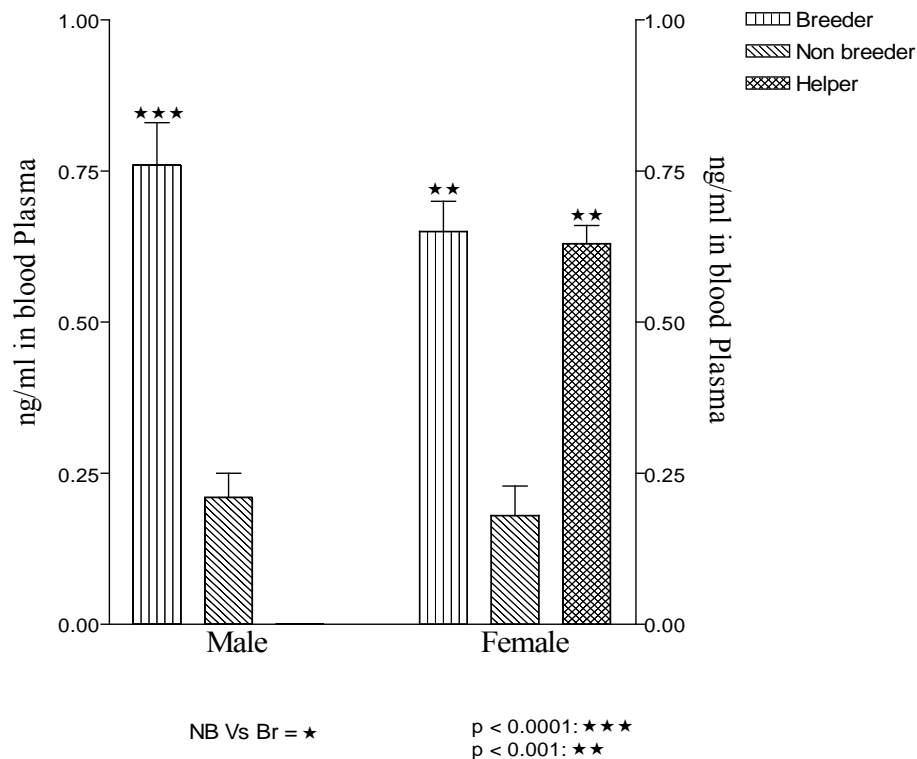


Fig. 5. Testosterone levels breeding and non breeding male, female and helper Jungle Babbler (*Turdoides striatus*).

Jungle Babbler (Fig. 6)

In Jungle Babblers, the progesterone levels were 0.3 ± 0.025 ng/ml of blood plasma and 0.15 ± 0.054 ng/ml ($F = 4.666$, $t = 6.175$, $P < 0.01$) in the breeding and the non-breeding males respectively. In the other sex, progesterone was 1.13 ± 0.066 ng/ml of blood plasma and 0.18 ± 0.042 ng/ml in the breeding and the non-breeding females respectively. In helper females it was 0.63 ± 0.033 ng/ml { $F = 694.8$, $t = (B \text{ Vs } NB = 37.20$, $P < 0.001$, $B \text{ Vs } H = 16.49$, $P < 0.001$, $NB \text{ Vs } H = 20.71$, $P < 0.001)$ } of blood plasma.

Body weight

In Table 1 and 2, the body weights of both the species of mynas and jungle babbler are given. The body weight during different phases of reproduction in Bank myna and Brahminy myna showed moderately significant variations. The body weight of female being more than male in both cases of mynas. Bank myna male showed 58.45, 71.29, 65.36 and 60.21 g { $F = 7.314$, $t = (PrB \text{ Vs } B = 1.96$, ns, $PrB \text{ Vs } PoB = 3.66$, $P < 0.01$, $PrB \text{ Vs } NB = 4.25$, $P < 0.001$, $B \text{ Vs } PoB = 1.705$, ns, $B \text{ Vs } NB = 2.28$, ns, $PoB \text{ Vs } NB = 0.58$) ns} during non-breeding, pre-breeding, breeding and

post-breeding respectively, while the females had 60.89, 73.12, 67.45 and 61.10 g { $F = 7.357$, $t = (PrB \text{ Vs } B = 1.86$, ns, $PrB \text{ Vs } PoB = 3.94$, $P < 0.01$, $PrB \text{ Vs } NB = 4.01$, $P < 0.001$, $B \text{ Vs } PoB = 2.08$, ns, $B \text{ Vs } NB = 2.15$, ns, $PoB \text{ Vs } NB = 0.068$) ns} during these phases respectively. In Brahminy myna, the body weight of males during the above said phases was 52.47, 62.31, 57.85 and 55.19 g { $F = 3.77$, $t = (PrB \text{ Vs } B = 1.46$, ns, $PrB \text{ Vs } PoB = 2.33$, ns, $PrB \text{ Vs } NB = 3.22$, ns, $B \text{ Vs } PoB = 0.87$, ns, $B \text{ Vs } NB = 1.76$, ns, $PoB \text{ Vs } NB = 0.89$) ns} respectively. The female Brahminy myna showed 54.25, 65.38, 60.12 and 56.73 g { $F = 4.86$, $t = (PrB \text{ Vs } B = 1.70$, ns, $PrB \text{ Vs } PoB = 2.79$, ns, $PrB \text{ Vs } NB = 3.60$, $P < 0.01$, $B \text{ Vs } PoB = 1.09$, ns, $B \text{ Vs } NB = 1.89$, ns, $PoB \text{ Vs } NB = 0.80$) ns} respectively during the different phases of breeding. In both mynas moderately significant increase is seen in body weights in both sexes of mynas in pre-breeding season while minimum of body weight is seen during the non-breeding season. In case of Jungle babblers which are social breeders no significant increase is seen in breeders and non-breeders males and females as well as in helper females. The body weights for male breeders and non-breeders being 61.4 and 56.77

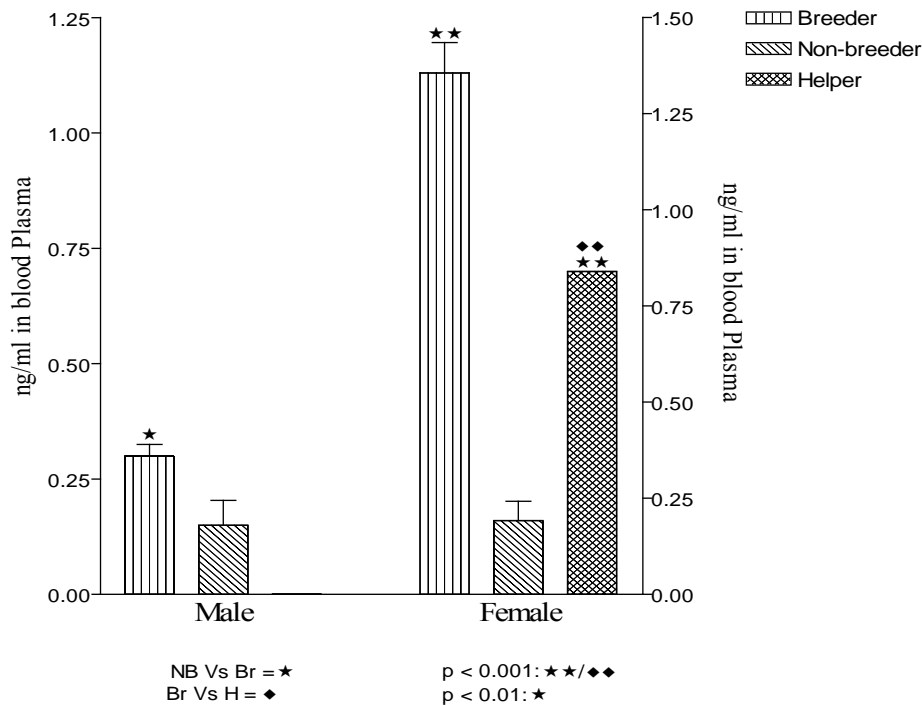


Fig. 6. Progesterone levels breeding and non-breeding male, female and helper Jungle Babbler (*Turdoides striatus*).

g ($F=1.027$, $t=1.452$, ns) respectively while the body weight of female breeders, non-breeders and helpers being 58.66, 54.0 and 58.14 g { $F=0.6562$, $t=(B\ Vs\ NB=1.04$, ns, $B\ Vs\ H=0.116$, ns, $NB\ Vs\ H=0.928$) ns} respectively.

(PrB=Pre-breeding, B=Breeding, PoB=Post-breeding, NB=Non-breeding, H=Helpers).

Discussion

In seasonally breeding species of birds, the changes in the external environmental factors like light, rain, etc. are known to trigger the cyclic reproductive activities (MURTON, WESTWOOD 1977, IMMELMAN 1971, PHILLIPS *et al.* 1985). The hormonal interplay and its influence on reproductive mechanism can be better understood by studying cyclic alterations in the levels of sex hormones over the reproductive cycle. In the present study two sex hormones, Testosterone and Progesterone are evaluated. Higher levels of plasma testosterone in male Mynas during pre-breeding as compared to the non-breeding phase of reproductive cycle (Fig. 1 and 2) indicate the initiation of breeding machinery. The occurrence of elevated plasma testosterone levels during breeding season is a well

documented fact and has been reported in Starlings (DAWSON 1983) Mocking birds (LOGAN, WINGFIELD 1995), Japanese Common Pheasant (SAKAI, SUSUMI 1986), Rose-ringed Parakeet (KRISHNAPRASADAN *et al.* 1988), Zebra finches (ADKINS-REGAN *et al.* 1990), Ostriches (DEGAN *et al.* 1994), Northern Pintail, (PENFOLD *et al.* 2000), White winged Crossbill (DEVICHE, SHARP 2001), Korean ring necked Pheasant (KIM, YANG 2001) and in the Ring Dove (FUSANI *et al.* 2001a). In the male White Crowned Sparrows peak testosterone levels occur during egg-laying (MORTON *et al.* 1990) while in Japanese quail it peaks during territorial aggression and other related reproductive behaviors (SCHLINGER, CALLARD 1990; WINGFIELD *et al.* 2001). But in the short-day seasonally breeding bird, the Emu (*Dromaius novaehollandiae*), the highest testicular testosterone concentrations were observed in the pre-breeding months and lowest in the non-breeding months, while the levels during the breeding season were intermediate (MALECKI *et al.* 1998). These reports compile that the peak testosterone levels occurs during various stages of breeding activities in different species of birds. It was also observed that the levels of testosterone were comparatively more in the solitary nester as that to the colonial

nester, revealing the fact that the solitary nesters are solely responsible for all the activities taking place during the breeding cycle, whereas in the colonial nester the whole colony is responsible for defending the territory as well as in search of food.

In the female Mynas low levels of circulating testosterone were measured during the pre-breeding, post-breeding and non-breeding phases. The higher testosterone levels in blood plasma of females during the breeding season only could be related to influence of this hormone in various breeding activities.

Plasma levels of testosterone were also determined in the breeding and the non-breeding individuals as well as helper females in Jungle Babblers, an altricial passerine with nearly continuous/long reproductive readiness indicate that reproductive behaviour including incubation and feeding is shared almost equally by both the parents or rather by the members of the whole flock (helpers). The gonadal development of these birds initiated in March and continued till November, which is reflected with the increase in the circulating testosterone levels in both sexes as well as in helpers. In males, different components of sexual behaviour are facilitated by direct and indirect action of testosterone. In social bird Mexican jays (*Aphelocoma ultramarine*), VLECK, BROWN (1999) have reported elevated testosterone during male-male competition for mate that decreases during parental or allo-parental care. Jungle Babbler though has feeding territory (ANDREWS 1968) do not show nesting territory and all the individuals of the flock take part in parental care. This is reflected in breeding males having non-significantly high testosterone levels compared to females in breeding state. The helper females also showed plasma testosterone almost equal to breeding females indicating their equal role in breeding activities except for egg laying. Testosterone has its fitness costs and influences aggressiveness and decreases the tendency to display parental care; hence testosterone is lower when necessity for parental care increases (VLECK, BROWN 1999). An elevated testosterone level in females of Macaroni and Gentoo penguins during post-copulation period has been associated with both sexes being involved in the nest defense (WILLIAMS 1992). TRAINOR, MARLER (2001) observed that in contrast to seasonally breeding birds, testosterone main-

tains parental behaviour in monogamous California mouse (*Peromyscus californicus*) by aromatizing testosterone to estrogen in brain. Testosterone mediates a negative correlation between parental behaviour and aggression in several seasonally breeding mammalian species (TRAINOR, MARLER 2001). This could be true for birds too.

In Jungle Babblers, where whole flock is involved in defending a single nest, lower titers of testosterone compared to other birds are noted. Female birds are generally less aggressive than their male species, which is probably related mainly to the lower androgen production of the ovary compared to the testes. In Jungle Babblers non-significant differences in the plasma testosterone levels are observed in the breeding males and breeding females as well as in the helper females suggesting that the nest defense, taking care of eggs, incubating them and protecting the young ones is a combined flock activity. Testosterone in male birds is known to stimulate nest building activity and the progesterone in females, to stimulate incubation behaviour by stimulating the release of endogenous prolactin from the bird's hypophysis (SEILER *et al.* 1992).

Higher progesterone levels in the female Mynas are associated with various breeding activities like courtship, incubation and brooding (WELTY, BAPTISTA 1990, JOHNSON 1986a). This is reflected by higher levels of plasma progesterone in female individuals of Bank Myna as well as Brahminy Myna during breeding season only (Fig. 3 and 4). The high levels of plasma progesterone can also be correlated with high 3 β -HSDH activity in the granulosa and the thecal cells of developing follicles and the extra-gonadal tissues of Mynas (SAPNA 2002), which contributes to the rising levels of plasma progesterone. A basal level of progesterone, one of the female sex hormone is known to occur in the male birds (JOHNSON 1986a). In the present study also, male birds of both species showed a constant lower level, i.e. 0.1 ng/ml of plasma progesterone all throughout the reproductive cycle (Fig. 3 and 4). However, in the third species Jungle Babbler, moderately increased levels of progesterone in males is noted which can be related to its social breeding habits where all the individuals of the flock are involved in incubation.

An increase in progesterone secretion has been claimed to play a significant role in mediating the transition from courtship to incubation behaviour in female ring doves (SILVER *et al.* 1974). LOGAN, WINGFIELD (1995) linked the progesterone rise to breeding behaviour in male and female mocking birds (*Mimus polyglottos*). In ovariectomised ring doves' administration of progesterone and estrogen facilitated nest building and incubation in response to the presence of nesting material and eggs respectively (CHENG, SILVER 1975). However, an average constant basal plasma progesterone level over the different phases of the reproductive cycle in males have been reported in several species of birds like Ring dove, *Streptopelia risoria* (SILVER *et al.* 1974), Chicken (FURR 1973), Pigeon (HAASE *et al.* 1976), Mallard duck (DONHAM 1979), White crowned sparrows (FARNER, MCCREERY 1979) and Mynas (SAPNA 2002).

Male Ringed Doves treated with Progesterone have been reported to show nest related pre-incubation behavioural patterns depending on experience (CHENG 1977; MICHEL 1977). There is a positive correlation between progesterone concentration and follicular development (SILVER *et al.* 1974). In females, the seasonal variation of progesterone is synchronous with the laying activity. In helper females intermediate levels of progesterone, i.e. higher than breeding males' but lower than breeding females supports their role in the parental activities. Oviduct development is influenced by progesterone, which induces the development of tubular glands in the magnum region of the oviduct (HUTCHISON 1975). It also results in defeathering and increased vascularity of brood patch in the breeding females (HUTCHISON 1975). In Jungle Babbler the plasma progesterone showed a pattern of variation different from that of

other hormones. WILLIAMS (1992) has reported that in both species and sexes of penguins, elevated plasma levels of progesterone occurred between arrival and early chick-rearing. The intermediate progesterone levels in helpers with subdued oviducal development thus indicate the involvement of helper in various reproductive activities except egg laying.

The current studies thus reveals few interesting facts: (1) That testosterone which is considered as a male hormone also influences female breeding activities especially in colonial and social breeders where as progesterone, a female hormone, as well as precursor of all steroid hormones, is present in blood plasma of male Mynas at low levels during all the phases of the seasonal reproductive cycle whereas at elevated levels in male Jungle Babblers indicating their involvement in breeding activities; (2) Higher levels of testosterone in male Brahminy myna is observed as compared to the other two species, as it's a solitary nester and the breeding pair carries out all the activities during the nesting period whereas comparatively intermediate and low levels in Bank myna and Jungle babbler respectively indicates that in colonial birds the colony is defended by all the breeding pairs and in social breeder, the load is shared by non-breeding individuals called helpers along with the breeding pair; (3) There is no species specific difference observed in the titers of progesterone in both mynas, while in Jungle babblers' progesterone levels of helpers are intermediate to that of breeders suggesting their allo-parental involvement.

Acknowledgements: The authors thank Indian Council of Agricultural Research (ICAR), New Delhi for financial assistance during which this work was carried out and Head, Department of Zoology, The M.S. University of Baroda for providing necessary facilities and encouragement.

References

- ADKINS-REGAN E, M. ABDELNABI, M. MOBARAK, M. A. OTTINGER 1990. Sex steroid levels in developing and adult male and female zebra finches (*Poephila guttata*). – *General and Comparative Endocrinology*, **78** (1): 93-109.
- ALI S. 1993. The Book of Indian Birds. Bombay Natural History Society, Bombay, 53-421.
- Andrews M. I. 1968. Ecology of the social system of Jungle babbler, *Turdoides striatus* (Dumont). Ph.D. Thesis submitted to the M.S. University of Baroda. Stranici
- BALTHAZART J, O. TLEM CANI and G.F. BALL 1996. Do sex differences in the brain explain sex differences in the hormonal induction of reproductive behavior? What 25 years of research on the Japanese quail tells us. – *Hormone and Behavior*, **30** (4): 627-661.
- BROWN J. L. 1987. Helping and communal breeding in birds. Ecology and evolution. Princeton University Press, Princeton, N.J. 354 p.
- CHENG M. F. 1977. Role of gonadotrophin releasing hormones in

- the reproductive behavior of female ring doves (*Streptopelia risoria*). – *Journal of Endocrinology*, **74** (1): 37-45.
- CHENG M. F., R. SILVER 1975. Estrogen-progesterone regulation of nest building and incubation behavior in ovariectomized ring doves. – *Journal of Comparative Physiology and Psychology*, **88**: 256-263.
- DAWSON A. 1983. Photoperiodic control of gonadotropin-releasing hormones secretion in sexually breeding birds. – In: *Neural Regulation in the Vertebrate Endocrine System*. Ed. By Prasada Rao and Peter Klwer. Academic/Plenum Publishers, NY, 85-100.
- DAWSON J. W. AND R. W. MANNAN 1991a. Dominance hierarchies and helper contributions in Harris Hawks. – *Auk*, **108**: 649-660.
- DEGAN A. A., S. WEIL, A. ROSENSTRAUCH, M. KAM AND A. DAWSON 1994. Seasonal plasma levels of luteinizing and steroid hormones in male and female domestic ostriches (*Struthio camelus*). – *General and Comparative Endocrinology*, **93** (1): 21-27.
- DELVILLIE Y, J. C. HENDRICK, J. SULON AND J. BALTHAZART 1984. Testosterone metabolism and testosterone-dependent characteristics in Japanese quail. – *Physiology and Behavior*, **33** (5): 817-823.
- DEVICHE P and P. J. SHARP 2001. Reproductive endocrinology of a free-living, opportunistically breeding passerine (white-winged crossbill, *Loxia leucoptera*). – *General and Comparative Endocrinology*, **123** (3): 268-279.
- DHARKUMARSINHI IME 1954. Birds of Saurashtra, India. Stranici
- DONHAM R. S. 1979. Annual cycle of plasma LH and sex hormones in male and female mallards (*Anas platyrhynchos*). – *Biology of Reproduction*, **21**: 1273.
- EMLÉN S. T. 1982a. The evolution of helping. I. An ecological constraints model. – *American Naturalist*, **119**: 29-39.
- EMLÉN S. T. 1982b. The evolution of helping. II. The role of behavioural conflict. – *American Naturalist*, **119**: 40-53.
- FARNER D. S. and B. R. MCCREERY 1979. Progesterone in male white crowned sparrows (*Zonotrichia leucophrys gambelii*). – *General and Comparative Endocrinology*, **37** (1): 1-5
- FURR B. J. A. 1973. Radioimmunoassay of progesterone in peripheral plasma of domestic fowl in various physiological states and in follicular venous plasma. – *Acta endocrinologica*, **72**: 89.
- FUSANI L, J. B. HUTCHISON, M. GAHR 2001a. Testosterone regulates the activity and expression of aromatase in the canary neostriatum. – *Journal of Neurobiology*, **49** (1): 1-8.
- HASSE E, E. PAULKE, P. J. SHARP. 1976. Effects of seasonal and social factors on testicular activity and hormone levels in domestic pigeons. – *Journal of Experimental Zoology*, **197**: 81.
- HAU M, M. WIKELSKI, K. K. SOMA, J. C. WINGFIELD 2000. Testosterone and year-round territorial aggression in a tropical bird. – *General and Comparative Endocrinology*, **117** (1): 20-33.
- HUTCHISON R.E. 1975. Effects of ovarian steroids and prolactin on the sequential development of nesting behavior in female Budgerigars. – *Journal of Endocrinology*, **67** (1): 29-39.
- IMMELMAN K. 1971. Ecological aspects of periodical aspects of reproduction. – In: *Avian Biology*, Vol I Ed. Farner and King. Academic Press. NY. London, 341-389.
- JOHNSON A. L. 1986a. Reproduction in females. – In: *Avian Physiology IVth edition* Ed. P.D. Sturkie, Springer-Verlag. NY, 403-431.
- JOHNSON A. L. 1986b. Reproduction in males. In: *Avian Physiology IVth edition* Ed. P.D. Sturkie, Springer-Verlag. NY, 432-451.
- KIM I. S., H. H. YANG 2001. Seasonal changes of testicular weight, sperm production, serum testosterone, and in vitro testosterone release in Korean ring-necked pheasants (*Phasianus colchicus karpowi*). – *Journal of Veterinary Medical Science*, **63** (2): 151-156.
- KRISHNAPRASADAN T. N., V. C. KOTAK, P. J. SHARP, R. SCHMEDEMANN, H. EBERHARD 1988. Environmental and hormonal factors in seasonal breeding in free-living male Indian Rose-Ringed Parakeets (*Psittacula krameri*). – *Hormone and Behavior*, **22** (4): 488-96.
- LOGAN C. A., J. C. WINFIELD 1995. Hormonal correlates of breeding status, nest construction, and parental care in multiple-brooded Northern Mocking birds, *Mimus polyglottos*. – *Hormone and Behavior*, **29** (1): 12-30.
- MALECKI I. A., G. B. MARTIN, P. J. O'MALLEY, G. T. MEYER, R. T. TALBOT, P. J. SHARP 1998. Endocrine and testicular changes in a short-day seasonally breeding bird, the emu (*Dromaius novaehollandiae*), in southwestern Australia. – *Animal Reproduction Science*, **53** (1-4): 143-155.
- MICHEL G. F. 1977. Experience and progesterone in ring dove incubation. – *Animal Behavior*, **25** (2): 281-285.
- MORTON M. L., L. E. PETERSON, D. M. BURNSAND, N. ALBAN 1990. Seasonal and age-related changes in plasma testosterone levels in mountain white crowned sparrows. – *The Condor*, **92**: 162-1173.
- MURTON R. K., N. J. WESTWOOD 1977. *Avian Breeding Cycles*. Clarendon Press Oxford, Oxford University Press. Oxford, U.K, 594 p.
- PENFOLD L. M., D. E. WILDT, T. L. HEROZOG, W. LYNCH, L. WARE, S. E. DERRICKSON, S. L. MONFORT 2000. Seasonal patterns of LH, testosterone and semen quality in the Northern pintail duck (*Anas acuta*). – *Reproduction Fertility and Development*, **12** (3-4): 229-235.
- PHILLIPS J. G., P. J. BUTLER, P. J. SHARP 1985. *Physiological strategies in Avian Biology*. 1 edition. Blackie and Sons Ltd. Glasgow and London, 112-139.
- RIETERS L. V., M. EENS, R. PINXTEN, G. F. BALL 2002. Seasonal changes in the densities of alpha (2) noradrenergic receptors are inversely related to changes in testosterone and the volumes of song control nuclei in male European starlings. – *Hormone and Behavior*, **44** (1): 63-74.
- SAPNA S. 2002. 'A comparative histoenzymological study of some tissues with reference to reproductive cycles in two species of birds'. PhD. Thesis submitted to The M. S. University of Baroda, Vadodara, India.
- SAKAI H., I. SUSUMI 1986. Annual cycles of plasma gonadotrophins and sex-steroids in Japanese Common Pheasants, *Phasianus colchicus veersicolor*. – *General and Comparative Endocrinology*, **63**: 275-283.
- SCHLINGER B. A., G. V. CALLARD 1990. Aromatisation mediates aggressive behavior in quail. – *General and Comparative Endocrinology*, **79** (1): 39-53.
- SEILER H. W., M. GAHR, A. R. GOLDSMITH AND H. R. GUTTINGER 1992. Prolactin and gonadal steroids during the reproductive cycle of the Bengalese finch (*Lonchura striata var. domestica*, Estrildidae), a non-seasonal breeder with biparental care. – *General and Comparative Endocrinology*, **88** (1): 83-90.
- SILVER R., C. REBOULLEUN, D. S. LEHRMA, H. H. FEEDER 1974. Progesterone during the reproductive cycle of male and female ring doves. *Endocrinology*, **94** (6): 1547-1554.
- SOMA K. K., A. D. TRAMONTIN, J. C. WINGFIELD 2000. Oestrogen regulates male aggression in the non-breeding season. – *Proceedings of Royal Society of London B. Biological Science*, **267** (1448): 1089-1096.
- STAUB N. L., MADELEINE DE BEER 1997. The role of androgens in female vertebrates. – *General and Comparative Endocrinology*, **108**: 1-24.

- TRAINOR B. C., C. A. MARLE. 2001. Testosterone promotes parental behavior in a monogamous mammal via conversion to estrogen. – *Proceedings of Royal Society of London B. Biological Science*, **269** (1493): 823-829.
- VLECK C. M., J. L. BROWN 1999. Testosterone and social and reproductive behavior in Aphelocoma jays. – *Animal Behavior*, **58** (5): 943-951.
- WALTERS J. R. 1990. Red-cockaded woodpeckers: A primitive cooperative breeder. – In: P. B. Stacey and W. D. Koenig (Eds.): *Cooperative Breeding in Birds: Long-Term Studies of Ecology and Behavior*. Cambridge Univ. Press, Cambridge, 67-102.
- WILLIAMS T. D. 1992. Reproductive endocrinology of Macaroni (*Eudyptes chrysolophus*) and Gentoo (*Pygoscelis papua*) Penguins. I. Seasonal changes in plasma levels of gonadal steroids and LH in breeding adults. – *General and Comparative Endocrinology*, **85** (2): 230-240.
- WELTY J. C., L. BAPTISTA 1990. *The Life of Birds*. IVth Ed. Saunders College Publishing, 19 p.
- WINGFIELD J. C., S. LYNN, K. K. SOMA 2001. Avoiding the 'costs' of testosterone: ecological bases of hormone-behavior interactions. – *Brain Behavior and Evolution*, **57** (5): 239-251.

Received: 14.10.2009

Accepted: 08.03.2010

Сезонни промени в концентрацията на прогестерон и тестостерон при три вида птици - *Acridotheres ginginianus* (Sturnidae), *Sturnus pagodarum* (Sturnidae) и *Turdoides striatus* (Muscicapidae), с различни размножителни стратегии

Б. Бхавна, С. Сапна, П. Геета

(Резюме)

Стереотипното репродуктивно поведение на птиците отразява променящата се интензивност на мъжките и женските полови хормони – тестостерон и прогестерон за двата пола. Повишените нива на тестостерона влияят върху ухажването и строенето на гнезда и при двата пола. От друга страна прогестеронът се появява при мъжките в малки количества по време на размножителния цикъл и е леко повишен по време на размножителния период. Настоящото изследване е върху нивата на тестостерон и прогестерон в мъжките и женските индивиди на три вида птици: *Acridotheres ginginianus*, *Sturnus pagodarum* (Sturnidae) и *Turdoides striatus* (Muscicapidae). Мъжките от *A. ginginianus* и *S. pagodarum* имат най-високи нива на тестостерон по време на размножителния период и сравнително ниски по време на пред-размножителния и пост-размножителния, и най-ниски в извън-размножителния. Женските показват по-висок тестостерон във връзка с участието си в ухажването и опазването на гнездата. Тези нива са ниски при колониално гнездящите в сравнение със самотно гнездящите птици. При *T. striatus* са открити незначителни нива на тестостерона в плазмата при размножаващите се мъжки и женски, както и при помощните женски птици. Прогестеронът – основният женски полов хормон, е на основни нива по време на целия размножителен цикъл при мъжките *A. ginginianus* и *S. pagodarum*, и с най-високо равнище при женските по време на размножителния сезон. Повишените нива на прогестерон при мъжките от *T. striatus* могат да се свържат със социалните размножителни навици доколкото мъжките са свързани с мътенето, а при женските промените са свързани с периода на снасяне.