JOURNAL OF EXPERIMENTAL ZOOLOGY 283:215–220 (1999)

Changes in Body Weight, Ovarian Growth, and Circulating Plasma Estradiol Level in Response to Programmed Photoperiods in Blackheaded Bunting, *Emberiza melanocephala*

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ABSTRACT Photosensitive female blackheaded buntings (*Emberiza melanocephala*) were observed at different photoperiodic schedules of increasing duration to study their effect on body weight, ovarian growth, and circulating plasma estradiol level. Continuous-light (24L:0D) and long-day (15L:9D) photoperiodic regimes showed a significant increase, followed by decrease, in body weight, ovarian growth, and circulating plasma estradiol level. The short photoperiod (8L:16D) showed no effect on body weight, ovarian growth, or circulating plasma estradiol level. During 24L:0D and 15L:9D birds became photorefractory and thus unable to maintain an elevated state of body weight, ovarian growth, or plasma estradiol level. On the other hand, the birds exposed to short photoperiod maintained their photosensitive state throughout the period. The peak attained during 24L:0D and 15L:9D treated groups showed little variation. The present findings indicate that long-day and continuous-light, when given for longer durations, causes photorefractoriness (a state in which birds are unable to respond to light) and thus suggest the involvement of endogenous circadian components in regulating reproduction and associated events during photoperiodic manipulation of light/dark cycles in female blackheaded buntings. *J. Exp. Zool. 283:215–220, 1999.* © 1999 Wiley-Liss, Inc.

Birds respond to annual change in daylength as an environmental cue, and its role in the control of reproductive activity and associated events involves an endogenous circadian component. Most of the literature available to date on avian photoperiodism focuses on temperate zone birds, while in the tropics and subtropics far fewer species have been investigated (see Tewary and Kumar, '82; Ravikumar and Tewary, '90; Kumar and Kumar, '93; Ravikumar et al., '95). It was previously thought that in the tropics and subtropics photoperiod may exert little influence on the regulation of metabolic and reproductive functions in birds (Immelmann, '71) the logic behind this being that there was very little annual variation in these regions. Later, studies from our laboratory revealed that the daylength has a more pronounced role in the control of reproduction and its associated events than had hitherto been assumed (Tewary and Kumar, '82, '83; Ravikumar and Tewary, '90; Kumar and Kumar, '93, '95; Tsutsui et al., '94). The effect of short and long photoperiods on reproductive rhythmicity in many avian species has been well studied (Farner and Follett, '66; Farner and Lewis, '71; Tewary and Kumar, '82; Kumar and Kumar, '95). The long daylength induction of gonadal growth and sex steroids has also been reported in several avian species (Wingfield et al., '91; Brain et al., '88; Tsutsui et al., '94; Ravikumar et al., '95). In birds, annual changes in gonadal weight as a parameter of gonadal activity are much smaller in the female than in the male (Farner and Moore, '85). Although there is much evidence for the photoperiodic regulation of reproductive functions in the male, not much is known about photoperiodic influences in females. The objective of the present investigation was to find out the variations in body weight, ovarian growth, and circulating plasma estradiol level in response to different programmed light/dark schedules. The experiments were also designed to investigate the role of circadian component(s) during photorefractoriness in photosensitive female blackheaded buntings, *Emberiza melanocephala*.

Grant sponsor: Council of Scientific and Industrial Research, New Delhi.

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Received 3 November 1997; Accepted 3 June 1998.

MATERIALS AND METHODS

Animals

The blackheaded bunting (*Emberiza melanocephala*) is a strong migratory species that arrives in the Indian subcontinent (25°18′N, 83°1′E) as the winter season approaches (September/October), remains through the winter, and returns to its breeding grounds at 40°N (West Asia and Eastern Europe) during spring season (March/April [Ali and Ripley, '83]). Adult female blackheaded buntings were captured locally near Varanasi in December 1994, when they were available in abundance. They were placed in an outdoor aviary for two weeks to acclimatize them. Food and water were supplied ad libitum at regular intervals. In the aviary, birds were freely exposed to natural daylength conditions.

Experimental design

In January 1995, forty-five female blackheaded buntings were brought to the laboratory and placed in small wire net cages (50 × 30 × 25 cm, 5 birds/cage) and all birds were exposed to short daylength photoperiodic schedules (8L:16D) for two months to ensure their photosensitivity. The birds were weighed and laparotomized periodically and shown to have maintained their normal body weight (19–21 g) and regressed ovarian growth (about 5–6 mg). These photosensitive birds were divided into three groups (15 birds/group) and were placed in programmed photoperiodic chambers under different photoperiodic regimes such as constant light (24L:0D), long (15L:9D), and short (8L:16D) photoperiodic schedules. Each photoperiodic chamber had three wire net cages (5 birds/cage) and was illuminated by a 20 W fluorescent tube with a light intensity of about 400 lux at perch level. Food and water were changed regularly only during light period. Five birds from each photoperiodic schedule were examined every month during a fixed period (between 15th and 20th of each month) to assess their body weight, ovarian growth, and plasma estradiol level. The reason for including 15 birds in each group was to analyse 5 birds every month. If a bird died, it was replaced by another bird of the same photoperiod to ensure the study of five birds per month. Research was conducted up until December 1995.

Sampling

Body weight was taken on single pan balance. Ovarian growth was assessed in situ by laparotomy, comparing the ovary size with standard sets of known ovarian weights. The rate of error using by this method is less than ±20% (Tewary et al., '83). The blood samples were collected in heparinized capillary tubes and centrifuged at 4°C (3000*g*). Plasma was separated, kept in Eppendorf tubes, and stored at –20°C until assayed for estradiol.

Radioimmunoassay of estradiol and statistical analysis

The circulating plasma estradiol level was measured using estradiol direct RIA kit (Biotecx Laboratories, Inc., Houston, TX). The kit was highly specific for estradiol. The cross-reactivity for 17β-estradiol was 100%. Data were analyzed using one-way analysis of variance test (ANOVA) followed by Newmann Keul's multiple range *t*-test at 1% and 5% significance levels (Brunning and Kintz, '77).

RESULTS

Effect of long days (15L:9D)

Photosensitive blackheaded buntings kept on long days (15L:9D) showed significant variations (Figs. 1 and 2) in body weight ($P < 0.001$, F-ratio 6.287, df 40, 9), ovarian growth ($P < 0.001$, F-ratio 249.707, df 40, 9), and plasma estradiol level ($P < 0.001$, F-ratio 959.132, df 10, 9). A significant increase in body weight ($P < 0.05$) was observed during April, May, and June, while in the rest of the period it was not significant. Ovarian growth also increased significantly during April, May, and June ($P < 0.01$), and July and August ($P < 0.05$) while it was not significant during other periods. The plasma estradiol level also showed significant increase during April, May, and June ($P < 0.01$) and July, August and September ($P < 0.05$), while during the rest of the period it was not significant. The peak was observed in May, when all three parameters were at maximal level and from June onwards it decreased until reaching its basal level in September.

Effect of continuous light (24L:0D)

Blackheaded buntings showed significant variations (Figs. 1 and 2) in their body weight ($P < 0.001$, F-ratio 17.247, df 40, 9), ovarian growth ($P < 0.001$, F-ratio (185.328, df 40, 9) and plasma estradiol level ($P < 0.001$, F-ratio 91.065, df 10, 9). A significant increase in body weight during April, May, and June ($P < 0.01$) and July ($P < 0.05$) was observed. Ovarian growth response was significant during April, May, June, and July ($P <$

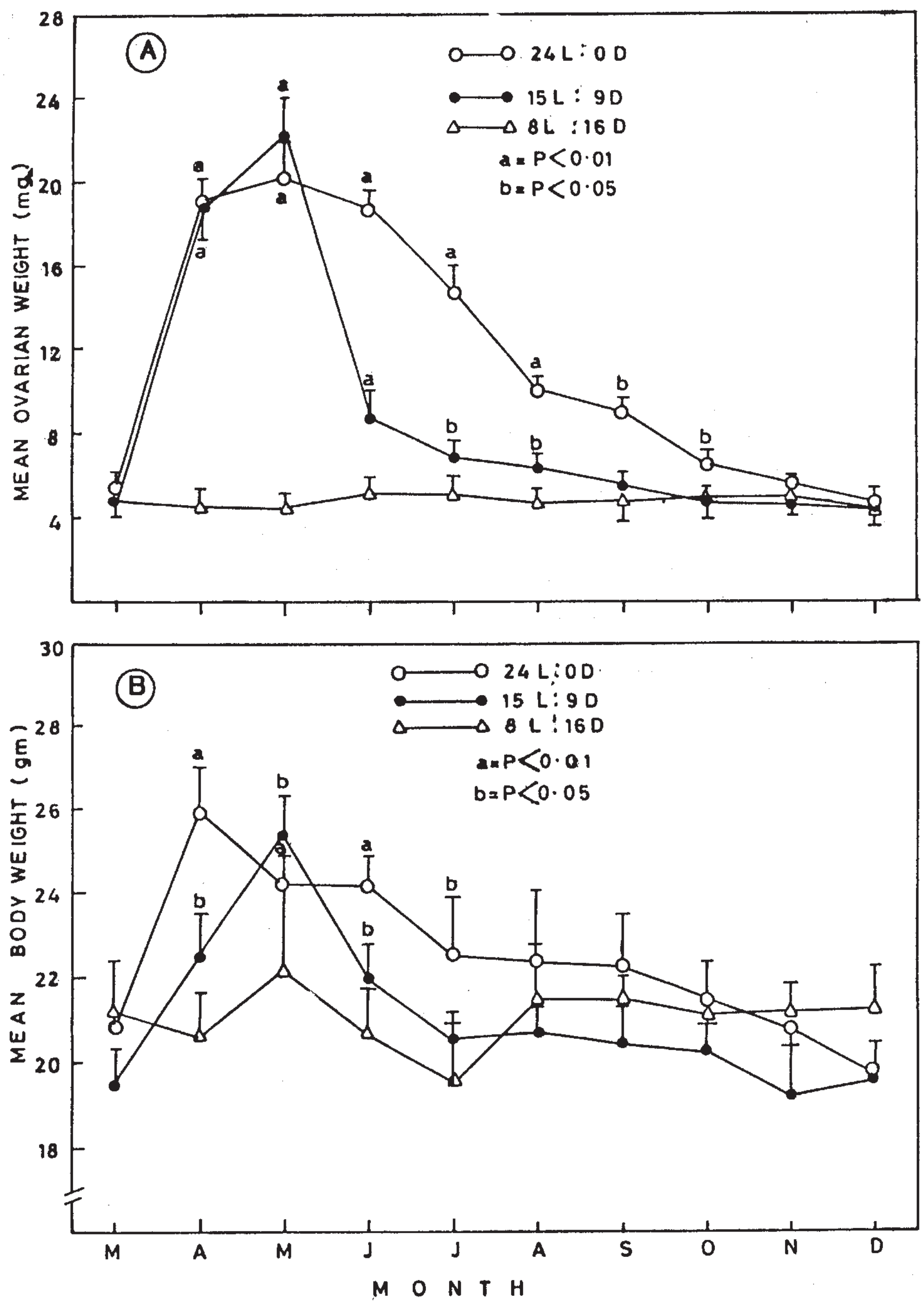

Fig. 1. Ovarian weight (**A**) and body weight (**B**) response in female blackheaded bunting under different light/dark (LD) schedules. Significant increase ($P < 0.001$) in body and ovarian growth during long and continuous light treatment was followed by regression, while short photoperiod showed no effect on body and ovarian growth. Vertical bar represents ± standard error of mean, n = 5 birds/observation.

0.01), and in August and September ($P < 0.05$). For the rest of the period it was nonsignificant. The plasma estradiol showed a significant increase during April, May, June, and July ($P <$ 0.01), while in August, September, and October ($P < 0.05$) and the rest of the period it was nonsignificant. The peak level of body weight, ovarian growth, and plasma estradiol was observed in April and from here onwards it decreased until reaching its basal level in September and then remained static throughout the rest of the experimental period.

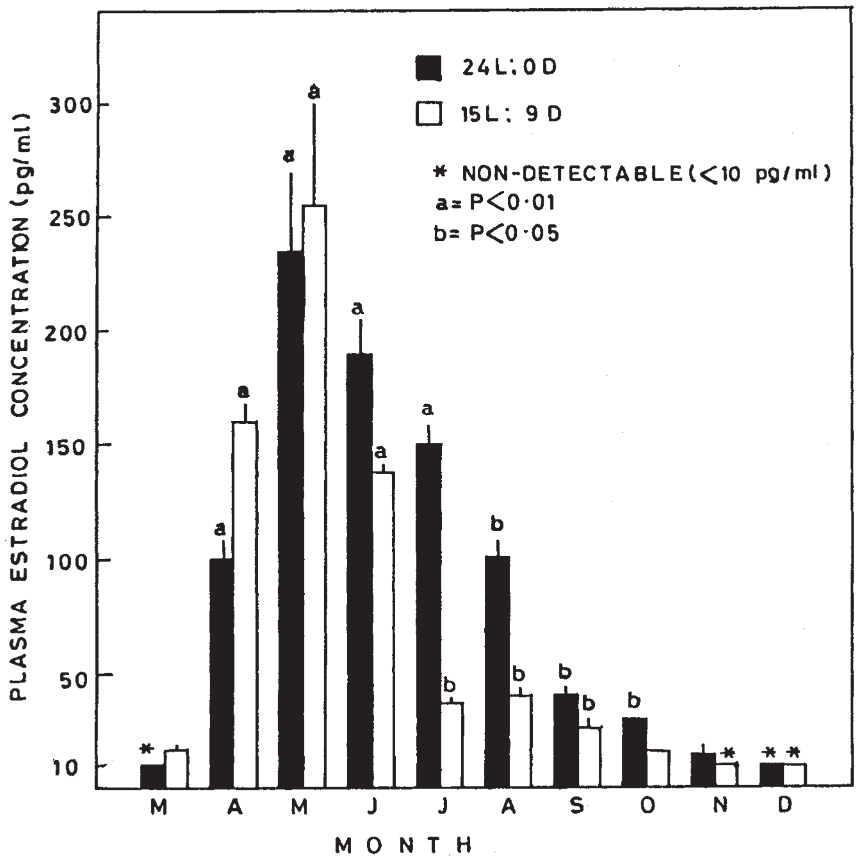

Fig. 2. Plasma estradiol concentration (pg/ml) in female blackheaded bunting. The long-day- and continuous-light-treated groups showed significant variation ($P < 0.001$) in plasma estradiol concentration. The plasma estradiol was undetectable during short photoperiod. Vertical bar represents ± standard error of mean.

Effect of short daylength (8L:16D)

Short daylength completely showed no effect (Fig. 1) on body weight (nonsignificant, F-ratio 1.664, df 40, 9) or ovarian growth (nonsignificant, F-ratio 0.545, df 40, 9) in blackheaded buntings. The plasma estradiol level was undetectable during this period.

Relationship between long day, continuous light, and short day illumination

Results indicate that long day (15L:9D) and continuous light (24L:0D) have a stimulatory effect on body weight, ovarian growth, and plasma estradiol level, while short day (8L:16D) is nonstimulatory. Birds attained their peak in May during long day and in April during continuous light. After attainment of peak level the body weight, ovarian growth, and plasma estradiol level started decreasing, suggesting that both photoperiods failed to maintain the elevated levels and thus gave way to a photorefractory state. On the other hand, short day has no effect on body weight, ovarian growth, or plasma estradiol level. When one compares the long day with continuous-light-treated birds, it is interesting to note that the photorefractory state was achieved later in continuous-light than in long-day-treated birds.

DISCUSSION

The present investigation indicates that female blackheaded buntings become photorefractory in response to either long day (15L:9D) or continuous light (24L:0D) if treated for longer time (more than two months). The photoperiodic responses of buntings, resemble those of several avian species in which long photoperiod causes full gonadal development followed by regression and onset of photorefractoriness. However, the initial response of long- and continuous-light treatments, where significant variations in body weight, ovarian growth, and plasma estradiol were at higher levels, shows consistency with earlier findings. Bernard and Ball ('97) showed that long day initially leads to increase in testosterone concentration in European starlings (*Sturnus vulgaris*). Long day induced increase in body weight in white crowned sparrows (*Zonotrichia leucophrys gambelii* [Schwabl et al., '88]); testicular development in red cross bill (*Loxia curvirostra* [Hahn, '95]); increase in LH secretion in prairie voles (Moffatt et al., '95); increase in body weight in Zebra finches (*Taeniopygia guttata* [Meijer et al., '96]); and elevated testosterone level and testicular weight in the Indian weaver bird (Tsutsui et al., '94). The induction of ovarian growth and increase in plasma estradiol level of female blackheaded bunting shows consistency with its male counterpart in which a high level of testosterone concentration and elevated testicular and body weight were observed during breeding season, when the daylength was more than 13 hours (Jain and Kumar, '95). Recently it was reported that the long day caused induction of ovarian mass and testicular growth in respectively female and male American tree sparrow (*Spizella arborea*) (see Reinert and Wilson, '96; Wilson and Reinert, '96). Follett et al. ('92) have shown that the cycles of short photoperiods are not inductive in Japanese quail (*Coturnix coturnix japonica*) but can be inductive if the daylength is increased. Continuous-light-(24L:0D)-treated birds showed ovarian growth followed by regression and onset of photorefractoriness, thus supporting earlier findings from our laboratory on male and female house sparrows (see Prasad and Tewary, '81; Tewary and Ravikumar, '89) and also comparing with those of north temperature zone birds (Hamner, '68; Schwab, '71). In contrast, the continuous illumination suppresses gonadal development in the subtropical spotted munia (*Lonchura punctulata* [Chandola et al., '75]). Short days failed to elicit any response in body weight, ovarian growth, or plasma estradiol level, thus showing consistency with female house sparrows (Tewary and Ravikumar, '89), female common canaries (Follett et al., '73), female redheaded buntings (Tewary and Tripathi, '83), rosefinches (Tewary and Dixit, '83), and male house sparrows (Tewary and Tripathi, '93). Our results are not consistent with those of some temperate zone birds in which continuous dark induced testicular development (Farner et al., '77), but it should be pointed out that at the time of transfer to continuous darkness these birds have already initiated testicular growth under natural photoperiod, which suggests that after transfer to continuous dark the gonadotrophic release persists for a while longer thus enabling an inductive effect.

A higher concentration of estradiol was associated with increased ovarian growth in blackheaded bunting. It has been reported that Japanese quail raised under long photoperiod have a higher concentration of estradiol (Brain et al., '88). The plasma estradiol level concentration, along with the increase in ovarian growth in buntings under different light/dark schedules supports the earlier findings of Brain et al. ('88). In the present investigation the plasma estradiol was undetectable during many phases, which could be expected, given that when the decrease in ovarian growth was at its basal level, very few mature follicles were present, which resulted in much less secretion of estradiol, hence the undetectability of plasma estradiol. In white browed sparrow weaver (*Plocepasser mahali*) the estradiol level was detectable only prior to ovulation, while in the rest of the phase it was undetectable (Wingfield et al., '91).

In conclusion, two points stand out from this study: (1) Female blackheaded buntings become photorefractory in response to long day and continuous light illumination. (2) Short daylength showed no effect on body weight, ovarian growth, or plasma estradiol level in female blackheaded buntings even when maintained for a longer time. It has been well established that short photoperiodic treatment is effective in dissipating the refractory period (Kumar and Tewary, '82; Silverin, '94). In the present investigation, after termination of experiments, the birds were treated with short photoperiods for 8 weeks to dissipate the refractory period, which caused photosensitivity. Moreover, these experiments have considerable implications regarding reproductive physiology of this species, since the same type of refractoriness occurs under prolonged photoperiodic treatments (15L:9D and 24L:0D) as occurs under natural conditions (unpublished data from our laboratory). The importance of the photorefractory period in the cyclicity of avian species is well established. What physiological, hormonal, and neural mechanisms are involved in the initiation, maintenance, and dissipation of photorefractoriness, however, is still a question of experimental debate, and further study is needed at the neuroendocrine level.

ACKNOWLEDGMENTS

Financial support from the Council of Scientific and Industrial Research, New Delhi, to P.D.T. is gratefully acknowledged.

LITERATURE CITED

Ali S, Ripley SD. 1983. Handbook of birds of India and Pakistan. vol. 10. Bombay: Oxford University Press.

Bernard DJ, Ball GF. 1997. Photoperiodic condition modulates the effect of testosterone on song control nuclei volumes in male European starlings. Gen Com Endocrinol 105:276–283.

Brain PC, Onagbesan OM, Peddie MJ, Taylor TG. 1988. Changes in plasma concentration of reproductive steroids in female Japanese quail (*Coturnix coturnix japonica*) raised on long or short photoperiod. Gen Com Endocrinol 69:174–180.

Brunning JL, Kintz BL. 1977. Computational handbook of statistics. 2nd edition. Glenview, Illinois, England: Scott Foresman and Co.

Chandola A, Pavanskar J, Thapliyal JP. 1975. Scotophotoperiodic responses of a subtropical finch (spotted munia) in relation to seasonal breeding cycle. J Interdiscipl Cycle Res 6:189–202.

Farner DS, Donham RS, Lewis RA, Mattocks PW, Darden TR, Smith JP. 1977. The circadian component in the photoperiodic mechanism of the house sparrow, *Passer domesticus*. Physiol Zool 50:247–288.

Farner DS, Follett BK. 1966. Light and other environmental factors affecting avian reproduction. J Anim Sci 25:90–118.

Farner DS, Moore MC. 1985. An assessment of the role of daylength in the control of the annual cycle of reproduction and molt in birds. In: Lofts B, Holmes WM, editors. Current trends in comparative endocrinology. Hong Kong: Hong Kong University Press. p 705–707.

Farner DS, Lewis RA. 1971. Photoperiodism and reproductive cycle in birds. In: Giese AC, editor. Photophysiology, vol 16. New York: Academic Press. p 325–370.

Follett BK, Hinde RA, Steel E, Nicholls TJ. 1973. The influence of photoperiod on nest building, ovarian development and luteinizing hormone secretion in canaries (*Serinus canarius*). J Endocrinol 59:151–162.

Follett BK, Kumar V, Juss TS. 1992. Circadian nature of the photoperiodic clock in Japanese quail. J Comp Physiol A 171:533–540.

Hahn TP. 1995. Integration of photoperiodic and food cues to time changes in reproductive physiology by an opportunistic breeder, the red cross bill, *Loxia curvirostra* (Aves: Canduelinae). J Exp Zool 272:213–226.

Hamner WM. 1968. The photorefractory period of the house finch. Ecology 49:211–217.

Immelmann K. 1971. Ecological aspects of periodic reproduction. In: Farner DS, King JR, editors. Avian biology, Vol. 1. New York: Academic Press. p 341–389.

Jain N, Kumar V. 1995. Changes in food intake, body weight, gonads and plasma concentrations of thyroxine, luteinizing hormone and testosterone in captive male buntings exposed to natural day lengths at 29°N. J Biosci 20:417–426.

Kumar BS, Kumar V. 1993. Photoperiodic control of annual reproductive cycle in sub-tropical Brahminy myna, *Sturnus pagodarum*. Gen Com Endocrinol 89:149–160.

Kumar V, Kumar BS. 1995. Entrainment of circadian system and variable photocycles (T-photocycle) alters the critical daylength for photoperiodic induction in blackheaded buntings. J Exp Zool 273:297–302.

Kumar V, Tewary PD. 1982. Photoperiodic testicular response and photorefractoriness in common Indian rose finch. Environ Control Biol 20:39–42.

Meijer T, Rozman J, Schulte M, Stach-Dreesmann C. 1996. New findings in bodymass regulation in zebra finches (*Taeniopygia guttata*) in response to photoperiod and temperature. J Zool 240:717–734.

Moffatt CA, Gerber JM, Blom JMC, Kriegsfeld LJ, Nelson RJ. 1995. Photoperiod effects on steroid negative feedback in female prairie voles (*Microtus ochrogaster*). Gen Com Endocrinol 100:92–95.

Prasad BN, Tewary PD. 1981. Response to subtropical population of *Passer domesticus* to constant illumination. Proc Indian Acad Sci (Anim Sci) 90:601–607.

Ravikumar G, Senthilkumaran B, Tewary PD, Goel AK. 1995. Circadian aspects of photoperiodic time measurement in a female house sparrow, *Passer domesticus*. J Biol Rhythm 10:319–323.

Ravikumar G, Tewary PD. 1990. Photorefractoriness and its termination in the subtropical house sparrow: involvement of circadian rhythms. Chronobiol Int 7:333–339.

Reinert BD, Wilson FE. 1996. The thyroid and the hypothalamus pituitary ovarian Axis in American tree sparrow (*Spizella arborea*). Gen Com Endocrinol 103:60–70.

Schwab RG. 1971. Circannual testicular photoperiodicity in the European starling in the absence of photoperiodic change. In: Menaker M, editor. Biochronometry. Washington: U.S. Nat Acad Sci. p 428–477.

Schwabl H, Schwabl-Benzinger I, Goldsmith AR, Farner DS. 1988. Effects of ovariectomy on long day induced premigratory fat deposition, plasma levels of luteinizing hormone, and prolactin, and molt in white crowned sparrows, *Zonotrichia leucophrys gamelii*. Gen Com Endocrinol 71:398–405.

Silverin B. 1994. Photoperiodism in the male great tits (*Parus major*). Ethol Ecol Evol 6:131–157.

Tewary PD, Dixit AS. 1983. Photoperiodic control of ovarian cycle in Rosefinch, *Carpodacus erythrinus*. J Exp Zool 228:537–542.

Tewary PD, Kumar V. 1982. Photoperiodic response of a subtropical migratory finch, the Blackheaded buntings (*Emberiza melanocephala*). Condor 84:168–171.

Tewary PD, Kumar V. 1983. Biochronometry of photoperiodically induced fat deposition in a migratory finch, the Blackheaded buntings (*Emberiza melanocephala*). J Zool (London) 200:421–430.

Tewary PD, Ravikumar G. 1989. Photoperiodically induced ovarian growth in the subtropical house sparrow (*Passer domesticus*). Environ Control Biol 27:105–111.

Tewary PD, Tripathi BK. 1983. Photoperiodic control of reproduction in female migratory bunting (*Emberiza bruniceps*). J Exp Zool 226:269–272.

Tewary PD, Tripathi RK. 1993. Photoperiodic testicular response of subtropical male house sparrow *Passer domesticus*. J Parasit Appl Anim Biol 2:93–96.

Tewary PD, Tripathi BK, Kumar V. 1983. Effect of exogenous prolactin on ovarian growth and fattening in the redheaded bunting, *Emberiza bruniceps*. Gen Com Endocrinol 52:315–318.

Tsutsui J, Saxena T, Saxena RN. 1994. Effects of season and photoperiod on the plasma and intratesticular testosterone levels in Indian weaver birds inhabiting the sub-tropical zone. J Exp Zool 270:281–287.

Wilson FE, Reinert BD. 1996. The timing of thyroid dependent programming in seasonally breeding male American tree sparrows (*Spizella arborea*). Gen Com Endocrinol 103:82–92.

Wingfield JC, Hegner RE, Lewis DM. 1991. Circulating levels of luteinizing hormone and steroid hormones in relation to social status in the cooperatively breeding white browed sparrow weaver, *Plocepasser mahali*. J Zool London 225:23–58.