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Seasonal Variation in Sensitivity of the Photoperiodic Response System in the Subtropical Tree Sparrow (*Passer montanus*)

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ABSTRACT

We investigated seasonal variations in response to photoperiods in subtropical tree sparrow (*Passer montanus*), by examining the effects of long and short days on stimulation of response and termination of refractoriness, respectively. We also assessed whether photoperiodic effects were sex dependent. In one study, wild sparrows were transferred in each month of the year to artificial long days (14L/10D) for 12 weeks. Birds transferred from November to March (female) or April (male) showed gradual increase in gonadal growth and darkening of bill color, while those transferred from April (female) or May (male) to July underwent gradual regression in the above responses. Moult in the wing primaries and body feathers progressed with gonadal regression and the birds transferred from April/May to October exhibited gradual increase and decrease in feathers moult. In another study, 6 weeks of short day (9L/15D) exposure could recover responsivity to long days in refractory birds. Male and female birds responded almost in similar fashion with some variations. Overall, our results show a gradual change over seasons in responsiveness of the endogenous response system to stimulatory effects of long day length. They suggest roles of both long and short day lengths in regulation of seasonal cycles in subtropical tree sparrows. *J. Exp. Zool. 317A:488–498, 2012.* © 2012 Wiley Periodicals, Inc.

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Most birds reproduce seasonally in order to coincide the birth of young ones when food is optimally present. They exhibit seasonality in various physiological and behavioral functions, including reproduction, moult, bill coloration, hormone levels, etc. (Deviche and Small, 2001; Dawson, 2007; Singh et al., 2010). Several environmental factors help in this timing. As change in photoperiod is entirely predictable at given latitude, both within and between years, it is used as a reliable cue to time the physiological preparations for three major life-history stages: reproduction, moult, and migration in a number of avian species (Hau et al., 2004; Bradshaw and Holzapfel, 2007). Thus, in most, if not all cases, day length is used as the primary cue in predicting the favorable time of the year for seasonal events (Helm et al., 2006). This involves interaction of day length with appropriate phases of the endogenous clocks during different times of the year (Trivedi et al., 2005; Kumar et al., 2010). Post-reproductively, many bird species stop responding to stimulatory day lengths, and undergo gonadal regression (Small et al., 2008). This is described as the photorefractory phase, which can qualitatively vary among photoperiodic species (MacDougall-Shackleton et al., 2006; Hahn and MacDougall-Shackleton, 2008; Hahn et al., 2009). Photorefractoriness is typically absolute in seasonally breeding songbirds (MacDougall-Shackleton et al., 2006), which is defined by either of the two criteria (Nicholls et al., '88). First, in species

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that become absolutely photorefractory the gonads will regress spontaneously when held on constant long days. In these birds, prebasic plumage moult proceeds with spontaneous gonadal regression without any decline in photoperiod. Second, once gonads have regressed, birds are completely unresponsive to very long days, even to 24 hr of light (MacDougall-Shackleton et al., 2006; Hahn and MacDougall-Shackleton, 2008). Birds recover from this state after they pass through decreasing days of autumn and winter in nature, which in laboratory is achieved by exposing long day photorefractory birds to 6–8 weeks of short days (Boulakoud and Goldsmith, '95; Kumar, '97). This suggests that both long and short days play significant roles in photoperiodic regulation of annual reproductive cycle, although species use them for different purposes. Annual reproduction is closely linked with moult in the annual cycles of the birds (Murphy, '96). Both events are such timed that they do not overlap; this saves huge costs since both are energy demanding (Hamborg et al., 2001). In majority of species, therefore, moult begins immediately after breeding (Dawson, '99). Further, photostimulation is required to induce moult but it also induces gonadal maturation and regression. It is unclear, however, whether day length has a direct effect on moult or consequential effect tied with reproduction (Dawson, 2007).

It is now recognized that birds inhabiting both high and low latitudes can discriminate between small changes in day length (Trivedi et al., 2006). Consequently, many low latitude avian species show annual reproductive cycles and photoperiodic responses that often resemble the seasonal breeding strategies of temperate birds (Wikelski et al., 2003). However, it still remains unsolved to what extent the mechanisms regulating seasonal reproduction in temperate and tropical birds are similar to or different from each other (Hau, 2001). Studies at relatively lower latitudes are fewer in view of large number of avian inhabitants at these latitudes. Therefore, critical field and laboratory experiments are required to ascertain the role of photoperiod in regulation of reproduction, moult, and migration in the birds inhabiting low latitudes with greater attention on local non-migratory species. Additionally, still fewer photoperiodic studies have been done on females, which may differ from males in relation to many aspects of reproduction related to physiology, morphology, and behavior (Ball and Ketterson, 2008). Thus, it is important to consider both sexes in the investigation of bird life history traits related to reproduction (Caro et al., 2009).

Therefore, we carried out present study at Shillong (25°34′N, 91° 53′E) on both sexes of widely distributed tree sparrow (*Passer montanus*; Sibley and Monroe, '90). We propose that with the data available on its conspecifics and related species spread across the world occupying different latitudes including temperate as well as tropical/subtropical photoperiodic environments, we would be able to examine the conservation of photoperiodic response mechanism. In our previous investigations on this species, we have revealed that tree sparrows at Shillong are photosensitive and use day length in regulation of their annual gonadal cycle (Dixit and Singh, 2011, our unpublished data). In the present study, we have specifically investigated whether (i) the photoperiodic control on reproduction and related seasonal events is affected by the change in responsiveness of the endogenous system to long days throughout in the year; (ii) male and female birds differently respond to day lengths; and (iii) both long and short days are important for photoperiodic regulation of gonadal cycles in the tree sparrow.

MATERIALS AND METHODS

Adult tree sparrows were captured from their wild habitat in and around the hills of Shillong, India using mist net and kept in an outdoor open aviary. This aviary is situated in the vicinity of our department in an open area surrounded by vegetation and receiving natural light and temperature conditions. Male and female birds were separated by performing laparotomy (surgical opening of abdominal wall between the last two ribs). For photoperiodic treatments, birds were kept in light proof wooden chambers (2.10 m × 1.20 m × 1.35 m or 2.50 m × 1.50 m × 2.10 m) illuminated by CFL bulbs (Philips Electronics India Limited, Kolkata, India) providing light of an intensity of ~400 lux at the perch level with automated control of light on and light off. Food and water were available ad libitum and were replenished only during the light phase of the cycle. Following experiments were performed using both the sexes of tree sparrows:

Experiment 1: Seasonal Variation in Photosensitivity

It began in January 2010 and continued till March 2011, and examined seasonal variation in photoperiodic stimulation under long days. Birds ($n = 6$–8 each sex) were captured from the wild in the middle of each month of the year 2010 and acclimatized in an outdoor aviary for 4–5 days before transferring them to long day length (14L/10D, close to the longest day length in June in Shillong; 25°34′N, 91°53′E) for 12 weeks. Male and female birds were housed separately in photoperiodic chambers (2.10 m × 1.20 m × 1.35 m). Observations on testicular volume, size of largest follicle, bill color, and body weight were made at the beginning and end as well as at the intervals of 4 weeks during the experiment. Also, primary feathers (PF) and body feathers (BF) were recorded as the measure of moult at the beginning and end and at intervals of 2 weeks during the experiment.

Experiment 2: Termination of Photorefractoriness

The second experiment determined whether short photoperiods given for a specific duration can break refractoriness in tree sparrow. In October 2009, when gonads are post-reproductively regressed, acclimated male and female sparrows for 4–5 days were exposed to a short day length (9L/15D) for 8 weeks. These birds (42 females and 45 males) were then exposed to long days (14L/10D) for 6 months. Periodic observations indicated gonadal growth-regression and photorefractoriness in all birds. They were then divided in to six groups for each sex. One group was maintained on 14L/10D and served as control for the next phase of the experiment. Remaining five groups, in each sex, were transferred to short day length (9L/15D, close to the shortest day length in December at Shillong) for 2, 4, 5, 6, and 8 weeks, after which they were returned to long days (14L/10D) for another 30 days to examine if they had shown photoperiodic stimulation. Observations on body mass and gonadal growth were made before transfer to a light regime and at the end of the light exposure.

The gonadal development was measured in terms of changes in testicular volume and follicle diameter. Briefly, the gonadal size was recorded in situ by performing laparotomy under local anesthesia using subcutaneous injection of 2% xylocaine (Astra-IDL Ltd., Bangalore, India) as per the procedure described by Kumar et al. (2001). Briefly, laparotomy was performed by surgical opening of abdominal wall between the last two ribs on the left side and gonad was located within the abdominal cavity with the help of a spatula. The length and width of the left testis was measured with respect to divisions on the graph paper using a calliper. Testicular volume was calculated using formula $4/3\pi ab^2$, where a and b denote half of the long (length) and short (width) axes, respectively. The ovarian growth was measured in terms of the diameter of the largest follicle. A regressed ovary with an indistinct follicle was assigned a follicular diameter of 0.3 mm to make the data statistically comparable with the stimulated follicles. Body weight was measured using a top pan balance to an accuracy of 0.1 g. The moult pattern was recorded by observations on primary wing feathers (called primaries) and BFs. For primaries, we followed scoring pattern as outlined by Boswell ('91) in a scale of 0–5 as per the following: 0 = worn or old feather; 1 = missing feather (just dropped); 2 = from a new feather papilla emerging up to one-third growth; 3 = new feather attaining two-third growth; 4 = new feather grown, but still the growth is not fully complete; and 5 = fully grown new feather. Thus, each PF can possess a minimum score of 0 and maximum of 5. As there are nine primaries on each wing, the maximum score per wing could be up to 45 ($9 \times 5 = 45$), and for each bird a maximum score of 90 (2×45) could be expected. Minimum score could be as low as 0. For recording body moult, whole body of the bird was divided into 12 different regions as follows: 1 = head; 2 = neck; 3 = shoulder; 4 = back; 5 = pelvic; 6 = caudal; 7 = throat; 8 = chest; 9 = abdomen; 10 = flank; 11 = shank; and 12 = sub-caudal. Each region could have a score of either 0 (no moult, fully grown or old feather present) or 1 (moult: no feather or new feathers emerging) and hence the total body moult score could be in the range of 0–12 (Budki et al., 2008). The bill color was scored in an index of 0–5: 0 = bill straw in color (S); 1 = bill straw in color but with a little tinge of blackness (ratio = SSS:B); 2 = bill slightly blackish in color (ratio = SS:B); 3 = bill straw and black in approximately 50:50 patches (ratio = S:B); 4 = bill black with very little straw patch left (ratio = S:BB); and 5 = bill completely black (B; Malik et al., 2004).

The data are presented as mean ± SEM. They were analyzed using two-factor nested ANOVA nesting the repeated measure within the bird and analyzing month as a between-subject factor followed by post hoc Bonferroni test, if ANOVA indicated a significance of difference. Three-factor nested ANOVA followed by post hoc Bonferroni test was also employed to compare responses between sexes. Gonadal growth rate (k) was calculated using the formula: $k = (\ln b - \ln a)/t$, where a and b are the initial and final testicular or follicular size, respectively, and t is time in weeks. We used Student's t-test to compare two means. Significance was taken at 95% confidence limit ($P < 0.05$).

RESULTS

Annual variations in day length at Shillong are shown in Figures 1A and 2A. Day length increases after its minimum in December till summer solstice and the longest day length of 13.44 hr is attained in June. It starts decreasing thereafter to reach to minimum (10.29 hr) by winter solstice in December. Thus, the day length varies in the range of 3:15 hr annually.

Experiment 1: Seasonal Variation in Photosensitivity

Results are presented in Figures 1 and 2. There was a gradual change in photosensitivity of the endogenous response system to stimulatory effects of long day length over the year for gonadal response. The birds showed gonadal growth and/or regression depending upon the month of their transfer to long day length. Two-factor nested ANOVA revealed a significant difference in testicular response to long photoperiod in the birds of different months over the year [month: $F_{11,192} = 81.354$, $P < 0.0001$; week (month): $F_{36,192} = 55.006$, $P < 0.0001$; Fig. 1B]. Birds transferred to long day length from November to April showed significant testicular response (December–April: $P < 0.0001$; November: $P < 0.05$) as compared to birds of October, exhibiting gonadal growth or its maintenance (April) followed by regression when observed over 12-week period. The rate of testicular growth and regression were faster in the March birds as compared to birds from November to February. The peak testicular volume (TV) was noticed after 8 weeks of transfer to long days in December ($TV = 62.10 \pm 5.15$ mm^3), January ($TV = 81.00 \pm 4.17$ mm^3), and February ($TV = 93.61 \pm 6.71$ mm^3) birds while it occurred earlier (only after 4 weeks of transfer) in March ($TV = 95.82 \pm 5.46$ mm^3) birds. Though a small increase in TV was observed in the April birds after 4 weeks ($TV = 68.55 \pm 6.93$ mm^3) of transfer to long days, gonadal stimulation did not persist and regression was evident thereafter. Further, all the birds transferred from May to July showed testicular regression. The rate of gonadal regression was significantly higher in May birds as compared to birds of June–July ($P < 0.0001$). The June birds showed significant gonadal regression ($P = 0.027$) when compared with July birds while no significant difference was observed between July and August birds. Further, birds of May and June showed a sharp decline in testicular size when observed after 4 weeks of

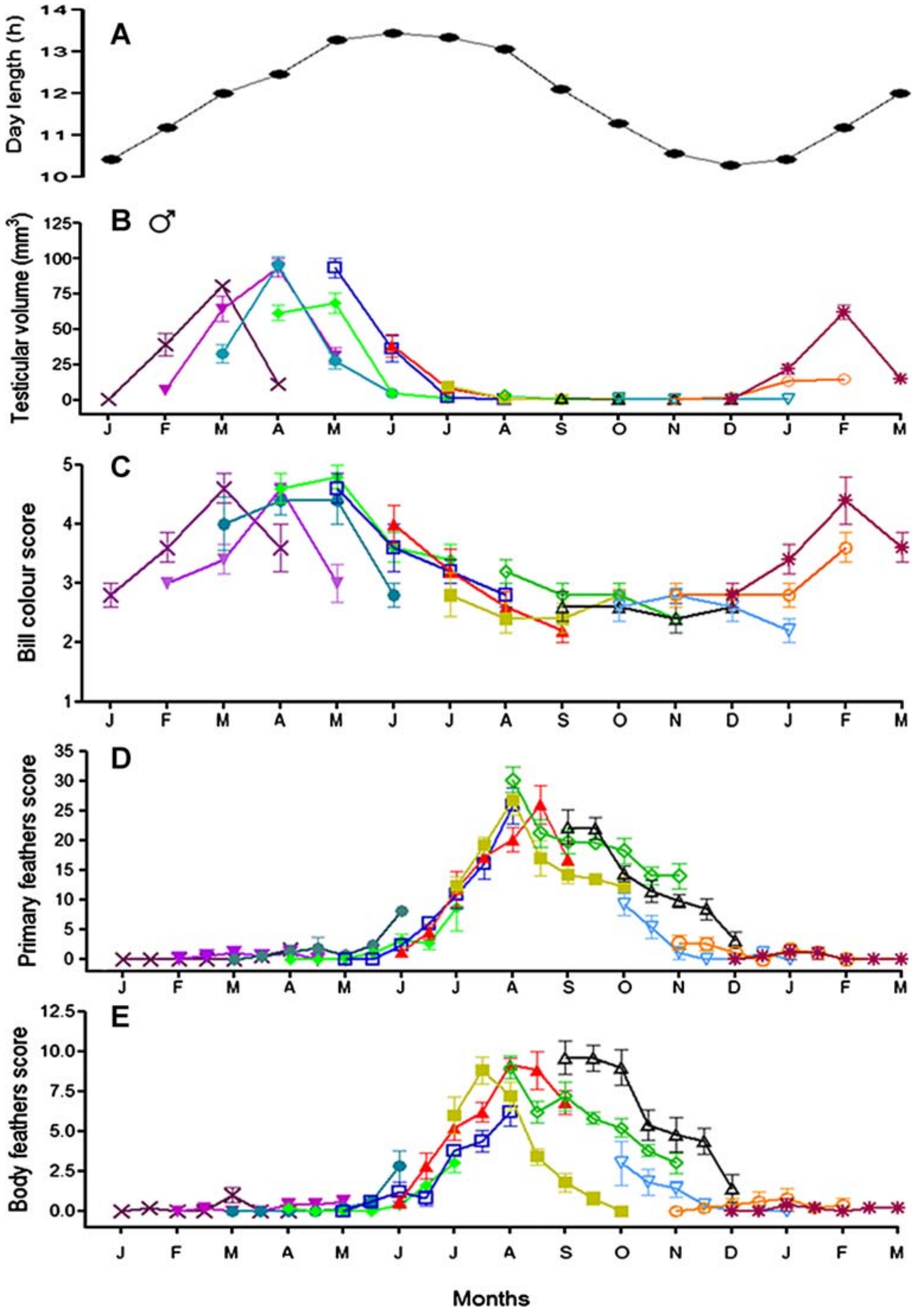

Figure 1. Seasonal variations in photosensitivity of endogenous response system for testicular (B), bill color (C), and moulting responses (D and E) in male tree sparrow. Results are presented as mean ± SEM. Different symbols and colors represent responses of birds in different months of transfer. Wild birds when transferred to artificial long daily photoperiod (14L/10D) in the middle of every month over the year underwent gradual changes in the above responses involving both increase and decrease depending upon the month of their transfer. Annual changes in day length at Shillong (A).

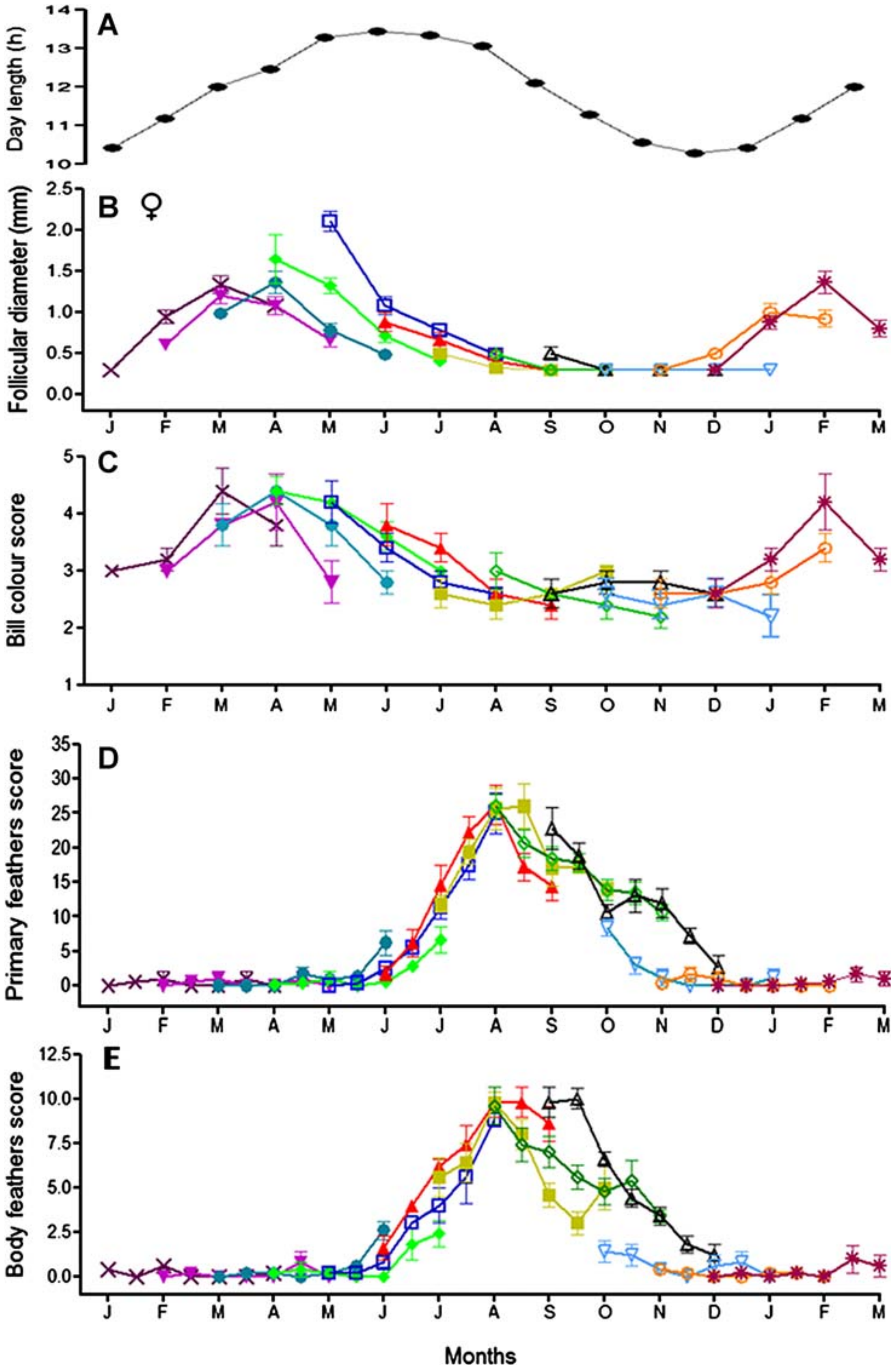

Figure 2. Seasonal variations in photosensitivity of endogenous response system for follicular (B), bill color (C), and moulting (D and E) responses in the female tree sparrow: Results are presented as mean ± SEM. Different symbols and colors represent responses of birds in different months of transfer. Wild birds when transferred to artificial long daily photoperiod (14L/10D) in the middle of every month over the year underwent gradual changes in the above responses involving both increase and decrease depending upon the month of their transfer. Annual changes in day length at Shillong (A).

transfer and the testes reached to minimum size in 8 weeks while testicular regression was slower in July birds that attained minimum testicular size earlier in 4 weeks only. On the other hand, birds of August–October maintained regressed testes throughout their exposure to long days. The first testicular response, though small, was observed in November birds only after 8 (TV $= 13.61 \pm 2.02$ mm^3) and 12 weeks (TV $= 19.99 \pm 1.78$ mm^3) of exposure to long day length. Further increase in gonadal response ($P < 0.0001$) was noticed in December birds when compared with the birds of September–November. They attained peak testicular growth on 8 weeks (TV $= 62.10 \pm 5.15$ mm^3) followed by gonadal regression suggesting that the birds become fully photosensitive by this time in nature after termination of photorefractoriness. Females transferred in different months exhibited significant follicular response [month: $F_{11,192} = 48.472$, $P < 0.0001$; week (month): $F_{36,192} = 18.879$, $P < 0.0001$; Fig. 2B] over the year. Birds transferred from November to March showed significant follicular growth ($P < 0.0001$), while follicular regression ($P < 0.0001$) was evident in the birds of April–July. Thus, the active period of gonadal function was 1 month shorter in females as compared to males. November birds showed follicular enlargement after 8 weeks of exposure to long days while it was evident only after 4 weeks in the birds of December–March. Further, peak follicular growth was attained earlier in February and March birds (i.e., after 4 weeks) as compared to December and January birds (i.e., after 8 weeks), and this was followed by ovarian regression. On the other hand, all the birds transferred from April to July underwent follicular regression while those transferred in August–October maintained their regressed follicles throughout their exposure to long days. A comparison using three-factor nested ANOVA revealed no significant difference in gonadal responses between male and female birds of different months exposed to long days over the year [sex (week (month)): $F_{36,288} = 0.873$, $P = 0.673$; week (month): $F_{11,288} = 0.069$, $P = 1.000$; month: $F_{11,288} = 9.024$, $P < 0.0001$].

Two-factor nested ANOVA revealed significant variations (darkening and lightening) in bill color of male birds over the year [month: $F_{11,192} = 18.528$, $P < 0.0001$; week (month): $F_{36,192} = 5.163$, $P < 0.0001$; Fig. 1C]. Changes in bill color ran almost parallel to changes in testicular size. Females exhibited almost similar trend of darkening and lightening of the bill color [month: $F_{11,192} = 11.976$, $P < 0.0001$; week (month): $F_{36,192} = 3.652$, $P < 0.0001$; Fig. 2C] as observed in the male birds except that the lightening started 1 month earlier and coincided with follicular regression. Further, a comparison using three-factor nested ANOVA revealed no significant difference in the bill color changes between male and female birds [sex (week (month)): $F_{48,384} = 0.529$, $P = 5.29$; month: $F_{11,384} = 24.84$, $P < 0.0001$ and week (month): $F_{36,384} = 8.742$, $P < 0.0001$].

Male birds exhibited significant variations in feathers moult [PF—month: $F_{11,360} = 146.679$, $P < 0.0001$; week (month): $F_{48,360} = 14.118$, $P < 0.0001$; Fig. 1D and BF—month: $F_{11,360} = 118.438$, $P < 0.0001$; week (month): $F_{48,360} = 10.099$, $P < 0.0001$; Fig. 1E, two-factor nested ANOVA] over the year. Though, a small but statistically insignificant moult in the primary and BFs was evident in March and April birds after 120 days of long days exposure, significant ($P < 0.0001$) moult was noticed only in May–October birds. On the other hand, moulting was not evident in November–February birds. Similar to males, moult in the wing primaries and BFs varied significantly over the year in females [PF—month: $F_{11,360} = 153.344$, $P < 0.0001$; week (month): $F_{48,360} = 12.569$, $P < 0.0001$; Fig. 2D and BF—month: $F_{11,360} = 149.019$, $P < 0.0001$, week (month): $F_{48,360} = 13.829$, $P < 0.0001$; Fig. 2E]. Females exhibited almost similar pattern of moult in their primary and BFs as observed in the males. They did not show moult when transferred to long days in November–February. A small but statistically insignificant moult was first evident in March and April birds only after 120 days of exposure to long days. However, significant moult was observed in the birds of May–October. They were found exhibiting different stages of moult during the course of experiment. Further, three-factor nested ANOVA showed no significant difference in the moult of wing primaries between male and female birds [sex (week (month)): $F_{84,672} = 1.213$, $P = 0.105$; month: $F_{11,672} = 365.730$, $P < 0.0001$ and week (month): $F_{72,672} = 23.63$, $P < 0.0001$), however, BF moult was found to be significant [month: $F_{11,672} = 304.767$, $P < 0.0001$; week (month): $F_{84,672} = 19.35$, $P < 0.0001$; sex (week (month)): $F_{84,672} = 1.549$, $P = 0.002$]. Birds of either sex did not show significant change in body weight over the year [male—month: $F_{11,192} = 1.784$, $P = 0.066$, $F_{36,192} = 0.915$, $P = 0.611$; female—month: $F_{11,192} = 1.545$, $P = 0.118$, week (month): $F_{36,192} = 0.732$, $P = 0.867$].

Experiment 2: Termination of Photorefractoriness

Birds showed testicular and ovarian growth followed by regression under long photoperiod (14L/10D) in 180 days. Regressed gonads were maintained in the birds of control group continued under 14L/10D indicating persistence of photorefractoriness in both the sexes (Fig. 3A and B). Photorefractory birds of either sex treated with 6 (male: $P = 0.0005$; female: $P = 0.001$, Student's t-test) and 8 (male: $P < 0.0001$; female: $P = 0.0002$) weeks of short days (9L/15D) showed significant redevelopment of their gonads when returned to long day. On the other hand, short day treatments of 2 (male: $P = 0.4767$; female: $P = 0.3400$), 4 (male: $P = 0.3739$; female: $P = 0.5942$), and 5 (male: $P = 0.1872$; female: $P = 0.2355$) weeks failed to break photorefractoriness in both the sexes as no significant change either in testicular size or in follicular diameter was observed. Further, there was no significant change in the body weight of either sex during the course of experiment.

DISCUSSION

It is evident from Figures 1–3 that both the sexes of the tree sparrow are photosensitive and exhibit post-reproductive

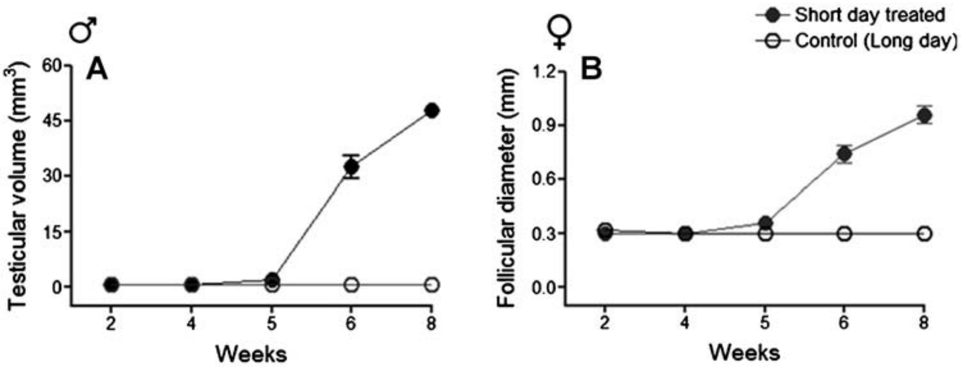

Figure 3. Termination of photorefractoriness in tree sparrow (**A**: male; **B**: female). Short day (9L/15D) treatments of 6 weeks and above were found to be sufficient to break photorefractoriness and the birds reacquired sensitivity to respond to long days.

refractoriness. The results further suggest seasonality in responsivity of the endogenous system to long days over the year. Seasonal responses in tree sparrow cycle between periods of photosensitivity and photorefractoriness. The initiation of gonadal growth is a long day phenomenon while the termination of photorefractoriness and consequent recovery of photosensitivity is a short day phenomenon in this species. Thus, both long and short days are important for photoperiodic regulation of seasonal responses in tree sparrow, although the bird uses them for different purposes. Further, there occurs a gradual increase and decrease in photosensitivity of the endogenous response system in both the sexes. First sign of small but significant increase in testicular size was observed in all the birds of November only after 8 ($TV = 13.61 \pm 2.02$ mm^3) weeks of transfer to long days, whereas birds of December–April responded only after 4 weeks of exposure. On the other hand, long days could not prevent the collapse of large gonads in May birds or re-initiate recrudescence of regressing gonads in June and July birds. The birds of August–October did not respond throughout their exposure to long days indicating that they remain photorefractory in these months. The above observations suggest that (i) the termination of breeding season in the tree sparrow is due to the development of photorefractoriness, and (ii) the photorefractoriness is followed by a recovery period, at the end of which birds regain their responsiveness to stimulatory day length. Thus, tree sparrows possess cyclicity in loss and gain in photosensitivity. The post-reproductive photorefractoriness in tree sparrow is very much similar to what has been observed in our previous studies on this species (Dixit and Singh, 2011) as well as on some other species (Dawson, ’91; Trivedi et al., 2006). In the present study, birds of May showed gonadal regression after 4 weeks of exposure to long days, that is, in June. Similar observations were recorded in the study of annual reproductive cycles of wild birds that showed gonadal regression and beginning of postnuptial moult in June before the day length declined in nature. Although, tree sparrows were not subjected to longer days including 24L:0D once gonads regressed spontaneously under long days in the laboratory or in the nature to check their responsiveness, we can conclude on the basis of above findings that they fulfil at least the first criterion of absolute refractoriness (MacDougall-Shackleton et al., 2006; Hahn and MacDougall-Shackleton, 2008). Photoperiodic responses of the tree sparrow are comparable to those of many temperate and subtropical photoperiodic avian species in which increasing day lengths in spring induce gonadal growth but fail to prevent regression of large gonads or to induce recrudescence of regressed gonads in summer months (Deviche and Small, 2001; Dawson, 2007; Singh et al., 2010). Seasonal changes in photosensitivity in the tree sparrow, thus, resemble to those of temperate zone gray partridge (*Perdix perdix*; Sharp et al., ’86), house sparrow (*Passer domesticus*; Dawson, ’91), subtropical weaver bird (*Ploceus philippinus*; Singh and Chandola, ’81), and brahminy myna (*Sturnus pagodarum*; Kumar and Kumar, ’91).

On comparing responses of the female tree sparrows with those of the males, it was found that both the sexes are photosensitive and show almost similar photoperiodic responses with the birds cycling between periods of photosensitivity and photorefractoriness. Though, significant gonadal growth ($P < 0.0001$) was evident in November birds in both the sexes, follicular regression ($P < 0.0001$) occurred 1 month earlier (i.e., in April birds) suggesting that the duration of sensitivity of the response system to long days for gonadal growth is 1 month shorter in females as compared to males. Although follicular size at the time of ovulation in feral tree sparrow is not known at present, it is evident from the observations of the present study and also from our unpublished data on the females that follicular stimulation in the captive birds was small. The diameters of largest follicles at the time of maximum ovarian stimulation in May in the nature and in captive conditions under 14L/10D were 2.35 ± 0.27 and

1.40 ± 0.05 mm, respectively. On the other hand, testes reached almost to the same size (TV = 97.33 ± 3.31 mm^3 in May) in the laboratory what was observed in the field (TV = 98.94 ± 4.31 mm^3 in May). Further, ovary does not grow to full breeding condition in tree sparrow under artificial photostimulation alone while testes reach to spermatogenic level under long days (our unpublished data). The substantial reduction in the ovarian response in the photoperiodic birds is due to failure of long daily photoperiods to induce vitellogenesis and the culminative stages of follicular development (Ball and Balthazart, 2002). Thus, the absence of final gonadal maturation is generally limited to one sex (Silverin and Westin, '95). From the proximate perspective, sex differences in the control of reproduction could be regulated via the response to photoperiod or in the relative importance and action of supplementary factors (such as temperature, food supply, nesting sites, and behavioral interactions) that adjust the timing of reproduction so that it is in step with local conditions (Ball and Ketterson, 2008). Therefore, it is quite understandable that besides the primary regulator (light in the photoperiodic species), some essential supplementary factors are required to bring about vitellogenesis and culminative stages of follicular development (Farner, '64), at least some of which are species specific and may involve environmental information, such as active mate or nesting materials or nest site, etc. (Farner and Lewis, '71). Further, food availability is a supplementary factor which can influence the timing of egg laying in birds (Svensson, '95).

The data presented in Figure 3A and B suggest that short day (9L/15D) treatment of 6 weeks is sufficient to break photorefractoriness and to develop sensitivity for long day photostimulation (14L/10D). These results clearly agree with the report that short days somehow dissipate refractoriness and make the birds photosensitive (Farner and Lewis, '71). The number of short days required to terminate photorefractoriness seems to be species specific. However, in general, 6–8 weeks of short day treatments terminate photorefractoriness by making the birds sensitive to photostimulation (Farner et al., '83). Tree sparrows, when transferred to long day length after 8 weeks of short day treatment, showed faster response (male: $P = 0.028$; female: $P = 0.04$) as compared to those treated for 6 weeks. Thus, longer the birds are exposed to short days, faster is the gonadal response upon transferring them to long days (Farner and Follett, '66; Hamner, '68). The results obtained from Experiments 1 and 2 on tree sparrow together suggest that the termination of photorefractoriness by short days is a phenomenon of continuous increase in photosensitivity from zero throughout the time of exposure to short daily photoperiods. The rate of recovery of photosensitivity in tree sparrow appears to be inversely proportional to day length as it was faster ($P < 0.0001$) in December (day length: 10 hr and 29 min) as compared to November (day length: 10 hr and 56 min) birds. The above observations on tree sparrow are in agreement with those on European starlings, *Sturnus vulgaris* (Dawson, '91). Photorefractory starlings when transferred from 18 to 6 L, 8, 11, or 12 L showed first signal of increase in LH (signaling the recovery of photosensitivity) after 4 weeks in the groups on 6 and 8 L, after 8 weeks in 11 L group and after 13 weeks in the 12 L groups suggesting an inverse relationship between the rate of recovery of photosensitivity and the photoperiod. It is unclear whether the rate of recovery of photosensitivity is dependent on the absolute length of short day, or on the magnitude of the decrease in day length. However, the rate of recovery of photosensitivity was found to be independent of day length in house finches (Hamner, '68). Tree sparrows showed faster response after 8 weeks of short day treatment when compared to 6-week treated birds suggesting that longer the birds are exposed to short days; the faster is the gonadal growth upon transferring them to artificial long days.

A comparison of seasonality in responsiveness to long days in tree sparrow with the annual gonadal cycle reveals a slow increase in photosensitivity and the termination of photorefractoriness in November and December. As the photoperiod experienced by the birds in the nature during December (day length: 10 hr and 29 min) to January (day length: 10 hr and 42 min) remains shorter than the threshold photoperiod (11 hr at Shillong; Dixit and Singh, 2011) for gonadal growth, the birds fail to show significant gonadal response despite being photosensitive. However, a slow but insignificant gonadal response was observed only when the increasing day length during spring falls above 11 hr/day in February (day length: 11 hr and 13 min). Thereafter, the rate of gonadal growth increases with increasing photoperiods from March to May (day length: 12 hr and 01 min to 13 hr and 28 min). Further, increase in photoperiod in June (day length: 13 hr and 44 min) induces spontaneous gonadal regression suggesting the onset and maintenance of photorefractoriness which is maintained till October (Dixit and Singh, 2011).

Wild tree sparrows of both the sexes exhibited significant moult in their wing primaries and BFs when transferred to long days from May to October that reached to peak in August birds. Further, increase and decrease in feathers moult were faster with the progression of month from May to July. It started declining in September and October birds and no moult was evident in the birds of November–April. The above responses were almost similar to annual moult cycle of the wild birds which showed moult in their primaries and BFs that progressed with gonadal regression extending from June to November with peak in August (Dixit and Singh, 2011). Wild tree sparrows of March and April showed beginning of moult in their primaries and BFs only after 8–10 weeks of exposure to long day length when the gonads in these birds were regressing. These results clearly suggest that the feather moult in the tree sparrow is somehow linked with gonadal regression when plasma levels of gonadal steroids also start declining (our unpublished data). The initiation of moult has been found to be linked to a decreased reproductive activity in a number of subtropical and in temperate avian species (Svensson and Nilsson, '97). It is not clear whether photoperiod has a direct effect on moult, or it is secondary consequence of photoperiodic stimulation of gonadal cycle and a physiological link between gonadal regression and moult, meeting the ecological requirement for moult to immediately follow breeding (Dawson, 2007).

Tree sparrows at different latitudes show some variations in their reproductive responses. In a study at Singapore (1°N), the breeding season of tree sparrow population lasted from January to mid-May (Wong, '83) suggesting that the bird population at this latitude become photosensitive earlier in nature. Further, a comparative study between both the sexes of the Malaysian population of tree sparrows (3°N) reveals a much longer breeding season lasting from December to June/July as compared to our study birds. They exhibited moult in their BFs throughout the year except in May with a high proportion of moulting adults during August–November. However, moult in the wing primaries and BFs progressed simultaneously in our study birds at 25°N. Thus, tree sparrow populations at 1°N and 3°N show longer breeding seasons and earlier acquisition of photosensitivity as compared to our birds at Shillong (25°N). On the other hand, tree sparrow populations at higher latitude (Poland: 52°N) have a breeding season from mid-April to early August suggesting attainment of photosensitivity later as compared to our birds (Wong, '83). It can be inferred from the above studies on various populations of tree sparrows at different latitudes that they attain photosensitivity slower, breed later and for shorter duration with the increasing latitudes with the exception of Malaysian tree sparrow at 3°N. Wild house sparrows at 27°N, when exposed to long day in months from January to April, showed significant gonadal growth and subsequent regression but long day could not prevent gonadal collapse in May birds in both the sexes and the gonads became fully regressed by the end of the experiment (Trivedi et al., 2006) as in tree sparrow at 25°N. The interspecific variation in breeding time of different avian species may be due to interspecific variation in their photoresponse systems (Hau, 2001; Coppack and Pulido, 2004). Thus, the photoperiodic response systems may be adaptively specialized to appropriately initiate and terminate reproduction at different times in different species. Alternatively, different breeding schedules may result from species with identical response systems living at different latitudes (MacDougall-Shackleton et al., 2006). The results obtained from Experiments 1 and 2 and subsequent analyses of the data suggest that despite relatively small annual photofluctuation in the subtropics, tree sparrows are capable of fine discrimination of photoperiodic information and use photoperiod to time their seasonal responses.

Bill color (Figs. 1C and 2C) in our birds runs almost parallel to increase and decrease in gonadal size under long days suggesting the possibility of its control by increasing and decreasing plasma levels of gonadal steroids, respectively. Further, both sexes exhibit almost similar pattern of bill color change with lesser intensity in females (Figs. 1C and 2C). This raises the possibility of its control by androgen in our study birds. This is supported by the report that the exogenous administration of testosterone induces darkening of the bill color in both the sexes of tree sparrow (Lalhmuoklien, '94). There are several lines of evidence suggesting that bill color is a testosterone-dependent trait in some birds including zebra finch (*Taenipygia guttata*; McGraw et al., 2003) and Eastern American goldfinch (*Spinus tristis*; Mundinger, '72). Ovaries synthesize and secrete adequate amount of androgen in domestic fowl (*Gallus gallus*), mallard (*Anus platyrhynchas*), killerdeer plover (*Charadrius vociferous*), and red wing black bird (*Agelaius phoeniceus*). Further, it has also been observed that ovaries of phalaropes (*Steganopus tricolor*) secrete more amount of androgen than testes (Hohn and Cheng, '67). The above reports suggest that almost similar pattern of bill color change in both the sexes of tree sparrows might be due to excessive secretion of androgen by the ovaries. However, further investigation is required to ascertain the role of sex steroids in control of bill color in tree sparrow. No significant change in body weight was observed in different experiments in the present study. This clearly follows the suggestions that fattening is generally lacking in non-migratory birds and if fattening occurs, it is only to a limited extent (Farner and Follett, '66). This accounts for minor but insignificant changes in the body weight in our birds.

In conclusion, our results show a gradual change over seasons in photoresponsivity of the endogenous response system and suggest roles of both long and short days in regulation of seasonal cycles in the subtropical tree sparrows. Seasonal responses of the bird cycle between periods of photosensitivity and photorefractoriness. Photorefractoriness can be terminated by exposing birds to at least 6 weeks of short days in laboratory or to shortening days of winter (November–December) in nature. Although, male and female birds show almost similar photoperiodic responses, they differ in the magnitude and duration of gonadal development. The present study, thus, provides evidence for the conservation of photoperiodic control mechanisms in the subtropical population of tree sparrow ensuring seasonal events to occur at the most suitable time of the year.

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LITERATURE CITED

Ball GF, Balthazart J. 2002. Neuroendocrine mechanisms regulating reproductive cycles and reproductive behaviour in birds. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT, editors. Hormone, brain and behaviour. Vol. 2. San Diego, CA: Academic Press. p 649–798.

Ball GF, Ketterson ED. 2008. Sex difference in the response to environmental cues regulating seasonal reproduction in birds. Phil Trans R Soc B 363:231–246.

Boswell T. 1991. The physiology of migratory fattening in the European quail, coturnix [PhD Thesis]. Bristol, UK: University of Bristol.

Boulakoud MS, Goldsmith AR. 1995. The effect on duration of exposure to short days on the gonadal response to long days in male Stralings (*Sturnus valgaris*). J Reprod Fertil 104:215–217.

Bradshaw WE, Holzapfel CM. 2007. Evolution of animal photoperiodism. Annu Rev Ecol Evol Syst 38:1–25.

Budki P, Rani S, Kumar V. 2008. Food deprivation during photosensitive and photorefractory life-history stages affects the reproductive cycle in the migratory red-headed bunting (*Emberiza bruniceps*). J Exp Biol 212:225–230.

Caro SP, Charmantier A, Lambrecht MM, Blondel J, Balthazart J, Williams TD. 2009. Local adaptation of timing of reproduction: Female are in the driver's seat. Funct Ecol 23:172–179.

Coppack T, Pulido F. 2004. Photoperiodic response and the adaptability of avian life cycles to environmental change. Adv Ecol Res 35:131–150.

Dawson A. 1991. Effect of daylength on the rate of recovery of photosensitivity in male starlings (*Sturnus vulgaris*). J Reprod Fert 93:521–524.

Dawson A. 1999. Photoperiodic control of gonadotropin-releasing hormone secretion in seasonally breeding birds. In: Rao P, Kluwer P, editors. Neural regulation in the vertebrate endocrine system. New York: Academic/Plenum. p 141–159.

Dawson A. 2007. Seasonality in a temperate zone bird can be entrained by near equatorial photoperiods. Proc R Soc B 274:721–725.

Deviche P, Small T. 2001. Photoperiodic control of seasonal reproduction: Neuroendocrine mechanisms and adaptation. In: Dawson A, Chaturvedi CM, editors. Avian endocrinology. New Delhi: Narosa Publishing House. p 113–128.

Dixit AS, Singh NS. 2011. Photoperiod as a proximate factor in control of seasonality in the subtropical male Tree Sparrow, *Passer montanus*. Front Zool 8:1.

Farner DS. 1964. The photoperiodic control of reproductive cycles in birds. Am Sci 52:137–156.

Farner DS, Donham RS, Matt KS, Mattocks PW, Moore MC, Wingfield JC. 1983. The nature of photorefractoriness. In: Mikami S, Homma K, Wada M, editors. Avian endocrinology environmental and ecological perspective. Tokyo: Japan Scientific Societies Press. p 149–166.

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

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

Hahn TP, MacDougall-Shackleton SA. 2008. Adaptive specialization, conditional plasticity, and phylogenetic history in the reproductive cue response systems of birds. Philos Trans R Soc Lond B Biol Sci 363:267–286.

Hahn TP, Watts HE, Cornelius JM, Brazeal KR, MacDougall-Shackleton SA. 2009. Evolution of environmental cue response systems: adaptive variation in photorefractoriness. Gen Comp Endocrinol 163:193–200.

Hamborg C, Sanz JJ, Lundberg A. 2001. Effects of latitude on the trade-off between reproduction and moult: a long-term study with pied flycatcher. Oecologia 129:206–212.

Hamner WM. 1968. The photorefractory period of the house finch. Ecology 49:211–227.

Hau M. 2001. Timing of breeding in variable environments: tropical birds as model systems. Horm Behav 40:281–290.

Hau M, Wikelski M, Gwinner H, Gwinner E. 2004. Timing of reproduction in a Darwin's finch: temporal opportunism under spatial constraints. Oikos 106:489–500.

Helm B, Piersma T, Vander Jeugd H. 2006. Sociable schedules: interplay between avian social and seasonal behavior. Anim Behav 27:245–262, 1215.

Hohn EO, Cheng SC. 1967. Gonadal hormones in Wilson's Phalarope (*Steganopus tricolor*) and other birds in relation to plumage and sex behavior. Gen Comp Endocrinol 8:1–11.

Kumar V. 1997. Photoperiodism in higher vertebrates: an adaptive strategy in temporal environment. Indian J Exp Biol 35:427–437.

Kumar V, Kumar BS. 1991. The development of photorefractoriness in termination of the breeding season in the tropical brahminy myna role of photoperiod. Reprod Nutr Dev 31:27–36.

Kumar V, Singh S, Mishra M, Malik S. 2001. Effects of duration and time of food availability on photoperiodic responses in the migratory male blackheaded bunting (*Emberiza melanocephala*). J Exp Biol 204:2843–2848.

Kumar V, Wingfield JC, Dawson A, Ramenofsky M, Rani S, Bartell P. 2010. Biological clocks and regulation of seasonal reproduction and migration in birds. Physiol Biochem Zool 83:827–835.

Lalhmuoklien. 1994. Breeding biology of certain avifauna in Imphal valley: Causa proxima analysis [PhD Thesis]. Manipur, India: Manipur University.

MacDougall-Shackleton SA, Katti M, Hahn TP. 2006. Tests of absolute photorefractoriness in four species of carduelines finch that differ in reproductive schedule. J Exp Biol 209:3786–3794.

Malik S, Rani S, Kumar V. 2004. Wavelength dependency of light-induced effects on photoperiodic clock in the migratory blackheaded bunting (*Emberiza melanocephala*). Chronobiol Int 21:367–384.

McGraw KJ, Gregory AJ, Parker RS, Adkins-Regan E. 2003. Diet, plasma caratenoids and sexual coloration in the zebra finch (*Taeniopygia guttata*). Auk 120:400–410.

Mundinger PC. 1972. Annual testicular cycle and bill color change in the Eastern American goldfinch. Auk 89:403–419.

Murphy ME. 1996. Energetics and nutrition of moult. In: Carey C, editor. Avian energetics and nutritional ecology. New York: Chapman and Hall. p 158–198.

Nicholls TJ, Goldsmith AR, Dawson A. 1988. Photorefractoriness in birds and comparison with mammals. Physiol Rev 68:133–176.

Sharp PJ, Massa R, Bottoni L, Lucini V, Lea RW, Dunn IC, et al. 1986. Photoperiodic and endocrine control of seasonal breeding in grey partridge (*Perdix perdix*). J Zool Lond A 209:187–200.

Sibley CG, Monroe BL, Jr. 1990. Distribution and taxonomy of birds of the world. London: Yale University Press.

Silverin B, Westin J. 1995. Influence of the opposite sex on photoperiodically induced LH and gonadal cycles in the willow tit (*Parus montanus*). Horm Behav 29:207–215.

Singh S, Chandola A. 1981. Photoperiodic control of seasonal reproduction in tropical weaver bird. J Exp Zool 216:293–298.

Singh J, Rani S, Kumar V. 2010. Presence of a conspecific renders survival advantages in the migratory redheaded bunting: test through the effects of restricted feeding on circadian response and survivorship. Chronobiol Int 27:111–127.

Small TW, Sharp PJ, Bently GE, Deviche P. 2008. Relative photorefractoriness, prolactin, and reproductive regression in a flexibly breeding sonoran desert passerine, the rufous-winged sparrow, *Aimophila carpalis*. J Biol Rhythm 23:69.

Svensson E. 1995. Avian reproductive timing—When should parents be prudent. Anim Behav 49:1569–1575.

Svensson E, Nilsson JA. 1997. The trade-off between molt and parental care: a sexual conflict in the blue tit? Behav Ecol 8: 92–98.

Trivedi AK, Rani S, Kumar V. 2005. Differential responses of the photoperiodic clock in two passerine birds possessing a strongly self-sustained circadian system. Chronobiol Int 22:801–806.

Trivedi AK, Rani S, Kumar V. 2006. Control of annual reproductive cycle in the tropical house sparrow (*Passer domesticus*): evidence for conservation of photoperiodic control mechanisms in birds. Front Zool 3:12.

Wikelski M, Hau M, Robinson WD, Wingfield JC. 2003. Reproductive seasonality of seven neotropical passerine species. Condor 105:683–695.

Wong M. 1983. Effect of unlimited food availability on the breeding biology of wild Eurasian tree sparrow in West Malaysia. Wild Bull 95:287–294.