Chapter I

Photoperiodic time measurement and photoentrainment of circadian activity rhythm

Introduction

Day length acts as a proximate environmental factor for seasonal reproduction in a variety of avian species (Kumar, 1997; Goldman, 2001; Dawson et al., 2001; Goldman et al., 2004; Trivedi et al., 2006; Dixit and Singh, 2011; Dixit and Sougrakpam, 2012). Various metabolic and reproductive activities, in most avian species, are regulated by day length (Rani et al., 2005; Hahn and MacDougall-Shackleton, 2008; Dixit and Singh, 2011). Some workers have attempted to study various aspects of avian photoperiodic control system (Dawson et al., 2001, 2007; Meddle et al., 2002; Coppack and Pulido, 2004). It is important to understand the mechanism(s) by which birds measure the day length to time their annual reproduction. The precision with which photoperiodic response occurs in nature clearly suggests the involvement of a fine photoperiodic time measurement scheme in most birds. The photoperiodic time measurement enables the birds to predict and prepare for seasonal changes in advance, ensuring their fitness in the seasonal environments (Bradshaw et al., 2004). It allow birds to recognize the time when to switch on (photoinduction) and switch off (photorefractoriness) their physiological mechanisms ensuring seasonal events to occur at the best-suited time of the year (Trivedi et al., 2006). Day length interacts with the endogenous clock and induces seasonal responses (Dawson et al., 2001). A circadian rhythm of photoperiodic photosensitivity (CRPP) mediates photoperiodic regulation of seasonal responses in many birds (Follett and Follett, 1981; Turek et al., 1984; Rani et al., 2002; Trivedi et al., 2006). CRPP responds to light in a phase-dependent manner (Rani et al., 2005). Such a concept was originally formulated by Büning (1936) and involves an external coincidence model.
(Pittendrigh and Minis, 1964). It predicts that photoperiodic induction occurs when the light coincides with the photosensitive or photoinducible phase of an entrained endogenous circadian rhythm. This model attributes a dual role to light, i.e. entrainer and inducer (Pittendrigh, 1966). Further, the reproductive activity in photoperiodic birds can be stimulated by a longer period of light followed by a shorter dark period (Trivedi, 2005). However, the light phase does not require to be continuous for longer period since interrupting the long dark period with an appropriately fixed short pulse of light will be sufficient to induce photoperiodic response that mimic the effects of a long day. One of the powerful methods to test the validity of external coincidence model is the use of night interruption experiments, in which two light pulses are given in 24 h light-dark cycle. These light periods consist of a main photophase of non-stimulatory duration (6-8 h) combined with dark phase which is interrupted by a short pulse of light once per cycle at a temporarily fixed point. These two light periods imitate the effect of corresponding long light period. The first light period, called the entraining light pulse (E-pulse) and given early in the subjective day, “entrains” the CRPP, so that the photoinducible phase (Φi) begins some “fixed” hours later in the day (Ball and Balthazart, 2003). These experiments were designed on the assumption that despite the quality of light being provided is similar at all times, its quantitative effects on gonadotropin secretion varies depending on where it falls in a 24 h cycle. Thus, the night interruption experiments are usually directed to show that it is not the duration of daily photoperiod that is important in inducing a photoperiodic response, but its relationship to the underlying cycle of photosensitivity is important. The night interruption experiments (often referred to as skeleton photoperiods) have been extensively used to examine the photoperiodic time measurement in a variety of avian and mammalian species including quail, white-crowned sparrows, house sparrows, house finches, green-finches, tree sparrows, sheep,
Entrainment programs the behaviour and physiology produced by endogenous circadian system in a proper timely manner. It requires the endogenous oscillators to be sensitive towards certain environmental cues as well as sensitivity towards others (Daan and Aschoff, 2001). Of all these cues, light has been a primary environmental factor in probing and unravelling the physiology of the circadian systems (Goldman et al., 2004; Rani et al., 2002). Light may affect the period of circadian oscillation. It modifies the shape or wave form and the level around which the oscillation moves. It also induces overt activity when the oscillation interacts with an endogenous threshold and remains as the oscillating variable as long as it is above the threshold (Aschoff, 1964). There is a limited range of entrainment, i.e. a limited range of T values around the circadian period (τ) where entrainment is possible. No visible influence on the circadian rhythm is observed when the period of a zeitgeber is outside the range of entrainment (Daan and Aschoff, 2001). Thus, the entrainment ability provides the proper phasing of the program to the sequence of the external changes. Furthermore, organisms can only entrain to synchronizers cycling with a period close to 24 h (Fuller and Fuller, 2002). Free-running condition arises if the period of entrainment is too long or too short thereby exceeding the range of entrainment and the circadian system cannot follow the Zeitgeber anymore. Since a strong Zeitgeber defines the rhythm of the clockwork, time is expressed as Zeitgeber time (ZT). In a rhythm of 12 h of light and 12 h of darkness (12L/12D), ZT00 is defined as lights on (beginning of the light phase) and ZT12 denotes to lights off (end of the light phase) (Jud et al., 2005).
Further, a circadian rhythm is defined as a regular event that is characterized by three parameters, viz. the mesor (M, rhythm adjusted mean), the amplitude (A, half of the difference between the minimum and maximum of the best fitting cosine function) and the acrophase (ϕ, the time of maximum of this cosine function with local midnight as ϕ reference) (Sahu and Shedpure, 2006). These parameters can be influenced by multiple factors such as alteration of day and night, changes in temperature, food availability and social factors (Houdas and Sauvage, 1971; Pati, 2001). There are various circadian characteristics that are associated with locomotor activity rhythm. The time difference between the entraining external and the displayed internal rhythm is called phase angle difference (Ψ) (Chandrashekaran, 2005). A phase shift (ϕ) is defined as the resetting of the organism’s internal rhythm in response to an external stimulus such as nocturnal light exposure (Pittendrigh, 1965). Such a phase shift can either result in a phase advance or a phase delay, where the former is the exact opposite of the latter. Circadian rhythms are sensitive to light in such a way that it advances or delays the phase of the rhythm depending upon the phase which is illuminated (Simpson and Follett, 1982). The period is the length of time necessary to complete one full cycle (e.g. the time required to go from peak to peak). The level of constant illumination to which a bird is exposed to affects the expressed period, represented by the letter, τ (tau) of the free-running rhythm.

The relationship between reproduction and day length is ultimately mediated through the neuroendocrine system, which perceives light and transduces signal that influences gonadal structure and their endocrine functions (Nakane and Yoshimura, 2010). The coincidence of light with the photoinducible phase of an entrained endogenous circadian rhythm converts thyroxin to triiodothyronin (Yasuo et al., 2005) in the brain leading to enhancement in cGnRH-I secretion (Yamamura et al., 2006) from hypothalamus that in turn stimulates release of gonadotropins from anterior pituitary
inducing gonadal growth and differentiation (Nakane and Yoshimura, 2010). The photostimulated gonads then increase the production of gonadal steroids such as testosterone and estradiol-17β that promote reproductive behaviour (Ball and Balthazart, 2002). They maintain gonadal function, ensure the negative feedback on higher levels of the reproductive axis, evoke secondary sexual characteristics (e.g. colour and shape of plumage, growth of the comb) and interrupt sexual behaviour (Johnson, 2000; Kirby and Froman, 2000). Testosterone is also associated with physiological changes that affect male sexual maturation (Beach and Inman, 1965; Ball and Balthazart, 2004) including the development of reproductive anatomy, the production of sperm and the onset of secondary sexual characteristics (Adkins-Regan, 2005) like song production and territorial aggression during breeding seasons (Hews and Moore, 1995; Hau et al., 1999; Terasawa and Fernandez, 2001). Increase in testosterone level can modulate locomotor activity rhythm in some avian species (Pohl, 1974; Gwinner, 1975; Turek and Gwinner, 1982; Subbaraj and Gwinner, 1985; Dixit and Singh, 2016). Further, the role of estrogens in vertebrates, including birds is also well documented (Di Cosmo et al., 2002; Dixit and Singh, 2011; Dixit and Sourakpam, 2012; Dixit et al., 2017b). They act at several levels to determine the behaviour and phenotype of the female, affecting the hypothalamic-pituitary unit and regulate the ovarian function and reproduction (Callard and Callard, 1987; Paolucci et al., 1999).

Extensive literature exists on avian breeding cycles and their control mechanisms with particular emphasis on the temperate birds and especially on the migratory species (Hau, 2001; Carey, 2009; French and Rockwell, 2011). Studies at relatively lower latitudes, particularly on local resident species, are few, though the number of avian inhabitants at these latitudes is large (Tewary and Dixit, 1986; Bhavna and Geeta, 2010; Dixit and Singh, 2011). The use of the night interruption experiments has been primarily
limited to the temperate zone photoperiodic species. There are very few reports on the use of such experiments on both the sexes of tropical/subtropical avian species. It is, therefore, logical to study more avian species and both sexes in order to test the generality of circadian rhythmicity in avian photoperiodic time measurement mechanism. Dixit and Singh (2011) have shown that the tree sparrow responds to some photoperiods (i.e. those above 10 h/day) but not to all. Further, its photoperiodic responses vary with the change in photoperiod. This suggests that the photoperiodic response system of this bird has efficiency to measure the photoperiodic time somehow with considerable degree of accuracy. Therefore, it was planned to study the mechanism(s) of photoperiodic time measurement and entrainment of the circadian locomotor clock in both the sexes of the subtropical tree sparrow (Passer montanus), a photoperiodic (Dixit and Singh, 2011) resident species distributed abundantly in the hilly regions of the North-East India at Shillong (Latitude 25°34’ N, Longitude 91°53’ E) to investigate (i) whether circadian rhythm is involved in photoperiodic time measurement during induction of gonadal growth and function, if yes, what is the duration of gonad stimulating photoinducible phase and (ii) whether illuminations of different parts of photoinducible phase (Φi) cause varying degrees of gonadal and circadian responses. Further, an attempt was also made to study the changes in circadian characteristics of activity rhythm in the tree sparrows subjected to various night interruption light-dark cycles.

**Materials and methods**

Wild tree sparrows were maintained in an outdoor aviary for 15 days before initiation of experimental procedures. In different experiments, birds were kept in light proof wooden chambers which were illuminated by light from compact fluorescent tubes providing white light (wavelength: 400 to 700 nm) of an intensity of 4.354 w/m² at the
perch level only in the light phase of various light-dark cycles. Following experiments were performed using tree sparrows:

1. **Night interruption experiment**

   This experiment was performed to investigate whether circadian rhythm is involved in photoperiodic time measurement during induction of gonadal growth and hormones. Photosensitive birds of both sexes were divided separately into eight experimental and a control groups (n= 6 each). The experimental groups were exposed to various night interruption light-dark cycles viz. G1- 6L/5D/1L/12D, G2- 6L/6D/1L/11D, G3- 6L/7D/1L/10D, G4- 6L/8D/1L/9D, G5- 6L/10D/1L/7D, G6- 6L/12D/1L/5D, G7- 6L/14D/1L/3D and G8- 6L/16D/1L/1D while the control group was maintained under 7L/17D for a period of 30 days. The increase in testicular size was considered as an index of gonadal response and was recorded by performing laparotomy under local anaesthesia using subcutaneous injection of 2% xylocaine (Astra-IDL Ltd. Bangalore, India) as per the procedure described in Kumar et al. (2001). The testis volume was calculated using the formula \( \frac{4}{3} \pi ab^2 \), where \( a \) and \( b \) denote half of the long (length) and short (width) axes, respectively. For the ovary, the diameter of the largest follicle was measured. The regressed ovary with indistinct follicles was assigned a follicular diameter (FD) of 0.3 mm to make the data statistically comparable with the stimulated follicles. The gonadal growth rate (k) was calculated using the formula: \( k = \frac{\ln b - \ln a}{t} \) where \( a \) and \( b \) denote initial and final gonadal size, respectively and \( t \) is time in days. Serum levels of testosterone and estradiol-17\( \beta \) were measured using ELISA in blood samples collected. The blood serum was used in the quantitative determination of testosterone and estradiol-17\( \beta \) concentrations by using ELISA kit (Testosterone- REF DF0002 and Estradiol-REF DK0003) procured from DiaMetra, Italy. The lowest detectable
concentration of testosterone and estradiol-17β that can be distinguished from the zero standards is 0.07 ng/ml and 8.68 pg/ml, respectively.

2. Investigation on locomotor activity

This experiment was done to investigate whether illuminations of different parts of photoinducible phase (Φi) changes characteristics of locomotor activity rhythm. Only male birds (n= 5) were used for this study. Activity movements of birds were measured using locomotor activity recording after first subjecting them to 12L/12D for 14 days and then transferring to various night interruption light-dark cycles such as 6L/6D/1L/11D; 6L/7D/1L/10D; 6L/8D/1L/9D; 6L/9D/1L/8D; 6L/10D/1L/7D; 6L/12D/1L/5D; 6L/14D/1L/3D; 6L/16D/1L/1D; 6L/17D/1L/0D for 7 days. For activity recording, each bird was kept separately in a specially designed activity cage (60 x 45 x 35 cm) that was furnished with two perches and mounted with a Napoleon miniature passive infrared detector (Maximum electronic Ltd., Israel) with a range of 16 m and wide angle (100°) field of view. Each sensor was connected to a separate computer channel of a window XP-compatible, and recording and analyses of the activity data (actograms) were done using the Chronobiology Kit (Release Version 1c, © 1998-2004) software program of the Stanford Software Systems, Stanford, USA.

Results

The results are presented in figures 1-3 and table 1.

1. Night interruption experiment

The results are presented in the figure 1(a-d). Both the sexes of tree sparrow responded in a similar fashion under various night interruption cycles. Birds of control group, exposed to (7L/17D), did not show gonadal response (Fig. 1a and c). However, a significant gonadal growth was observed in all the birds of G1 (male: P=0.0019; female:
P=0.0335), G2 (male: P=0.0011; female: P=0.0062), G3 (male: P=0.0048; female: P<0.0001), G4 (male: P=0.0044; female: P<0.0001), G5 (P=0.0009; female: P=0.0007), G6 (male: P<0.0001; female: P=0.0012) and G7 (male: P=0.0002; female: P<0.01, Student’s t-test). The birds under these groups behaved as though they had been exposed to stimulatory photoperiod (long day). On the other hand, no significant response was observed in both the sexes of tree sparrow included in G8 (male: P=0.3699 and female: P=0.1019). The birds in this group behaved as though they were under nonstimulatory photoperiod (short day). One-way ANOVA showed significant variations (male: F_{8,52}=14.86, P<0.0001; female: F_{8,52}=26.39, P<0.0001) in gonadal size of birds under different night interruption cycles. There was an increase followed by a decrease in gonadal size with increase in the time of interruption of the dark phase in the stimulatory groups (Fig. 1a and c). There were significant variations in gonadal growth among the birds of stimulatory groups of both the sexes. Largest testes and ovarian follicles were observed in G4 (33.72±5.56 mm^3) and G5 (0.94±0.06 mm), respectively which were significantly greater (P<0.001) when compared with other stimulatory groups. Further, gonadal growth rate varied significantly (male: F_{8,52}=20.13, P<0.0001, Fig. 1a; female: F_{8,52}=30.06, P< 0.0001, Fig. 1c; One-way ANOVA) among various light-dark cycles. The increase in serum levels of testosterone (F_{8,26}=12.06, P<0.0001) and estradiol-17β (F_{8,26}=39.49, P<0.0001) ran almost parallel to testicular and follicular size, respectively (Fig. 1b and d). Significant (P<0.05) increase in testosterone and estradiol-17β levels was observed in the birds of groups (G1-G7) when compared to C or G8. The testosterone level started increasing in G1 (0.337±0.039 ng/ml), reaching to its maximum (peak) in G4 (0.67±0.091 ng/ml) and decreased thereafter, reaching to a minimum value in G8 (0.103±0.054 ng/ml). Similar trend was exhibited by estradiol-17β. It started increasing in G1 (20.86±5.274 pg/ml), attained peak in G5 (66±4.015 pg/ml) and reached
to a minimum in G8 (3.267±3.267 pg/ml). However, clear sexual difference in the attainment of peak gonadal size and steroid levels were noticed that occurred in G4 in males and in G5 in females.

2. Investigation on locomotor activity

Results are presented in figures 2-3 and table 1. Birds, when exposed to light-dark cycle of 12L/12D, showed entrainment of their activity rhythm with activity confined mainly in the light phase (Fig. 2a-c). One-way ANOVA revealed significant difference in the activity period among the groups ($F_{9,69} = 129.5$, $P<0.0001$; Fig. 3a). The resting period was also found to be significant ($F_{9,69} = 129.5$, $P<0.0001$; Fig. 3b). The ratio of activity and rest periods ($\alpha/\rho$) was found to be significantly ($P<0.001$) higher in the cycles of 6L/9D/1L/8D and 6L/10D/1L/7D when compared to other cycles (Fig. 3d). There was gradual increase followed by decrease in $\alpha/\rho$ with increase in time of 1 h light interruption of 17 h dark phase. Significant variation was observed in the circadian activity under various night interruption light-dark cycles ($F_{9,200} = 3.863$, $P<0.0001$; Fig. 3c). The activity movements per day was found to be significantly ($P<0.05$) higher under the light-dark cycles of 6L/7D/1L/10D, 6L/8D/1L/9D, 6L/9D/1L/8D, 6L/10D/1L/7D and 6L/14D/1L/3D when compared to 6L/6D/1L/11D, 6L/12D/1L/5D, 6L/16D/1L/1D and 6L/17D/1L/0D. However, no significant difference in daily activity was observed among the birds of 12L/12D, 6L/7D/1L/10D, 6L/8D/1L/9D, 6L/9D/1L/8D, 6L/10D/1L/7D and 6L/14D/1L/3D (Fig. 3c). Furthermore, mean activity/hour for 24 h differed significantly among various light-dark cycles (hour: $F_{23,4800} = 138.4$, $P< 0.0001$; photoperiod: $F_{9,4800} = 20.84$, $P<0.0001$ and interaction: $F_{207,4800} = 7.293$, $P <0.0001$; Two-way ANOVA; Fig. 2c). It increased gradually with increase in time of 1 h light interruption of 17 h dark phase until it reached to peak in the cycle of 6L/10D/1L/7D and declined gradually thereafter to reach the minimum value under 6L/17D/1L/0D (Fig. 2c). The analysis,
using cosiner regression curve based on activity movement, revealed sinusoidal rhythmicity in all the night interruption cycles (Fig. 2c). The peak activity was observed to occur earliest in 6L/14D/1L/3D (Acrophase: 0.22±0.28 h; Mesor: 80.56±5.32 and Amplitude: 101.5±7.57; $R^2 = 0.7227$) while it was latest in 6L/6D/1L/11D (Acrophase: 5.42±0.35 h; Mesor: 63.28±4.61 and Amplitude: 70.47±6.53; $R^2 = 0.6278$; Table 1).

Further, the activity onset was recorded to be earlier with the increase in the time of interruption of 17 h dark phase. Significant group differences in activity onset ($\Psi_o$: $F_{9,69} = 33.59$, $P<0.0001$) and offset ($\Psi_e$: $F_{9,69} = 16.63$, $P<0.0001$; One-way ANOVA) were noticed when we compared the start of the activity with light ON and the end of activity with the light OFF, respectively (Fig. 3e and f). The $\Psi_o$ were significantly ($P<0.001$) higher in the cycles of 6L/12D/1L/5D, 6L/14D/1L/3D, 6L/16D/1L/1D, 6L/17D/1L/0D when compared to those of other light-dark cycles and exhibited phase advance (+$\Psi$; Fig. 3e). However, $\Psi_e$ were significantly ($P<0.001$) high in the light-dark cycles of 6L/7D/1L/10D, 6L/8D/1L/9D, 6L/9D/1L/8D and 6L/10D/1L/7D when compared to other night interruption cycles and showed phase delay (-$\Psi$; Fig. 3f). Furthermore, there was an abrupt change of 360° (from longer hour of -$\Psi_e$ to longer hour of +$\Psi_o$) in the cycles from 6L/12D/1L/5D to 6L/17D/1L/0D (Figs. 2 and 3).

**Discussion**

The results obtained from the above experiments suggest that the tree sparrow possesses a time measuring device that utilizes an endogenous circadian rhythmicity for inducing gonadal and hormonal functions and regulating activity behaviour. They can be interpreted on the basis of Bünning hypothesis and external coincidence model of photoperiodic time measurement (Bünning, 1973; Pittendrigh and Minis, 1964). Tree sparrows of the control group maintained under short day (7L/17D) did not show gonadal response (Fig. 1a-d). When the short photoperiod of 7 h was given to the
experimental birds in two split periods (6 h primary photoperiod and 1 h light pulse), the birds in some groups behaved as though they had been exposed to long day. It is important to note here that the total amount of light (7 h) given in any night interruption cycle was shorter than the photoperiodic threshold (about 11 h per day) for initiation of gonadal response in the tree sparrow (Dixit and Singh, 2011). Thus, though the birds experienced equal total amounts of light (7 h) and darkness (17 h) under all light regimes, they did not show similar responses. These results clearly indicate the importance of position of light in an endogenous circadian rhythm of photosensitivity in inducing photoperiodic gonadal responses. The position of light pulse relative to photoinducible phase of CRPP was found to determine photoperiodic gonadal response in tree sparrow. The photoinduction occurs if and only when light coincides with the photoinducible phase (Pittendrigh, 1972; Bunning, 1973; Tewary and Dixit, 1983). On the other hand, light falling in the nonphotoinducible phase produces a short day effect (Sansum and King, 1975; Kumar and Tewary, 1984). Further, the brief light pulse administered at various time points (between 11-22 h after respective dawn) during night, besides a basic 6 h main photoperiod, was not equally effective in inducing gonadal response. Light pulse given after 11, 12, 13, 14, 16, 18 and 20 h after the onset of primary photophase led to significant testicular and follicular growth (Student’s t-test) and gonadal steroid secretions while interruption after 6 and 22 h were ineffective (Fig. 1a-d). Such results strongly favour the presence of fine time measuring device in tree sparrows which enables them to discriminate annual photofluctuation (3 h and 15 min at Shillong) by using endogenous circadian rhythm to time their reproductive functions. The rates of gonadal growth and steroid secretions increase with increase in time of interruption of the photoinducible phase (Φi) reaching to maximum when interrupted at 14 h in male (Fig. 1a and b) and 16 h in female (Fig. 1c and d) and decline thereafter to
attain minimum levels at 22 h. This indicates that the photoresponse system of sparrow possess differential photosensitivity in different positions of Фi.

Our results are consistent with the reports on some other avian species in showing faster gonadal response with increase in the time of light interruption of Фi up to certain time points (Wilson and Siopes, 1976; Follett and Millette, 1982; Rani and Kumar, 1999). They are interesting as they do not suggest the re-entrainment of the photoperiodic clock by an inducing light pulse falling 20 h or more after dawn. In contrast, the bimodality due to re-entrainment of the photoperiodic clock was suggested (Lofts, 1975) in the temperate population of tree sparrow when light pulse fell 14 h after the onset of the first light period. The circadian system is clearly multi oscillatory and constitutes several independent circadian oscillators (Singh et al., 2002). Also, the pacemaker(s) in birds regulating various circadian rhythms appear to be different and may have distinct properties, although they may show close phase relationship (Kumar, 2001; Kumar et al., 2002). Although the exact duration of photoinducible phase has not been traced out in our study bird, it extends from about 11 to 21 h point (Fig. 2). Thus, the photoinducible phase seems to be of about 10 h duration. Further, photoinducible phase seems to begin 11 h after dawn that corresponds to the critical day length for gonadal growth in this species (Dixit and Singh, 2011). Similar to gonadal size, there was an increase followed by decrease in serum levels of sex hormones (testosterone and estradiol-17β) with the increase in time of 1 h interruption of 17 h night. This indicates that a brief exposure to light pulse during the night was sufficient to influence the biochemical and neural events underlying the photoperiodic control of neuroendocrine-gonadal activity in our birds under gonadostimulatory cycles. The Hong Kong population of tree sparrow also showed increase and decrease in testosterone levels when exposed to a total of 6.25 h of light in a day and the dark period was interrupted by a
light pulse of 15 minutes (Lofts, 1975). Furthermore, gonadal size and gonadal steroids were found running almost parallel to each other in tree sparrows as reported in some other species (Heath et al., 2003; Williams, 2012; Dixit and Sougrakpam, 2012). An increase in serum levels of gonadal steroids with increase in gonadal size (Fig. 1a-d) suggests that larger gonads produce more hormones as a result of enhanced gonadal activity in terms of steroids production. This is expected as the photostimulated gonads act as endocrine organs secreting these steroids required for development of secondary sexual characters and reproductive behaviours for successful reproduction. Testosterone in male birds facilitates reproductive physiology and behaviours needed for sperm production, development and maintenance of the cloacal protuberance, mate acquisition, territorial defence and song production (Smith et al., 1997) while estradiol-17β, in females, act at several levels to determine the behaviour and phenotype, growth and development of secondary sexual characters and synthesis of vitellogenin (Paolucci et al., 1999). The male tree sparrow displays a circadian pattern in locomotor activity with a strong preference of restricting activity during the light phase and arresting it in the dark phase as is true for a day active bird. The preference of an organism in being active during the day or night has an evolutionary significance since it enables organism to perform certain activities at the time of the day or the year when it is most advantageous. Birds, when exposed to 12L/12D, showed entrainment of their activity rhythm with the activity confined mainly in the light phase (Fig. 2a-c). The adaptive value of a circadian rhythm lies in its being rightly phased in relation to the external environment. This is achieved by the synchronization of the circadian oscillators with zeitgebers. The cosiner regression curve of circadian activity exhibited a distinct sinusoidal rhythmicity in all the night interruption cycles (Fig. 2c). Though the birds under different cycles exhibit almost similar general pattern of activity, they showed cycle dependent differences in the
characteristics of activity-rest cycle (Figs. 2-3 and Table 1). The interruption of 17 h dark period by 1 h of light at different circadian times gradually shifted the activity to the dark phase with constant change in phase angles between the light ON and OFF (Fig. 3e,f). These substantial phase shifts in steady state of the rhythm ultimately led to gradual lengthening of activity period until it reached to a peak in the birds under 6L/9D/1L/8D and 6L/10D/1L/7D cycles (Fig. 3a). The activity period started declining thereafter with further increase in circadian time of interruption of the dark phase until it reached to minimum in sparrows under 6L/17D/1L/0D (Fig. 3a). There was gradual increase in phase delay (-Ψ) when the supplementary 1 h of light pulse fell at 12, 13, 14, 15, 16, and 17 h after dawn in the 17 h dark phase. In contrast, gradual increase in phase advance (+Ψ) was noticed in case of interruptions at 19, 21, 23 and 24 h. This is in agreement with the report that the supplementary light pulses, applied early in the subjective night, cause significant phase delay (-Ψ) and is accepted as the terminator of the simulated skeleton photoperiod or as “new dusk” while those applied late in the subjective night and early in the subjective day cause significant phase advance (+Ψ) and function as the new initiator or “new dawn” (Pittendrigh, 1965; Saunders, 1977). The position of the phase jump depends on the duration of the main photoperiod, becoming later and later relative to light on (ZT 00) as the larger component is increased (Saunders, 1977). Further, there was an abrupt 360° change from phase delay to phase advance when 1 h pulse of light was applied at the middle of the subjective night as in 6L/12D/1L/5D cycle in our study bird.

In conclusion, our observations of the subtropical population of the tree sparrows suggest that their entrained, endogenous circadian rhythm of photosensitivity has a distinct photoinducible phase which, when illuminated, results in a photoperiodic gonadal response of gonadal growth and steroid secretion. This phase extends for 11-21
h after dawn. They display circadian rhythms in their locomotor activity that can be entrained to 24 h complete and skeleton light-dark cycles. Further, the sparrows exposed to these cycles exhibit changes in various circadian characteristics.