Studies on the effects of photic and non-photic cues on circadian and seasonal responses in the tree sparrow, *Passer montanus* (L.)

Abstract

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Abstract

A habitat on earth experiences daily and seasonal changes in sunlight (duration, spectrum and intensity), food availability or abundance, climate (temperature, rainfall, etc.) and social factors in the environment. Habitats are diverse and to cope with such diversity, birds possess physiological mechanisms that allow them to temporally coordinate energetically demanding stages in their life, for example, reproductive events with conditions in the environment. Birds plan their life-history stages in such a way so as to live successfully in a seasonal environment. Seasonal timings of life-cycle activities like reproduction, moult and migration in a given environment require preparations. Of all the life-history stages in an organism, breeding is predominantly restricted within a definite time window, to guarantee greater food availability for raising the young and maximize reproduction. Thus, appropriate timing of breeding is a challenge and an important component of reproductive success for almost all birds that live in seasonally fluctuating environments. Seasonality in reproduction and associated events is, thus, a compulsory adaptation for survival in many species. It is represented by the initiation-termination-reinitiation of physiological processes and is species specific. The annual life-history stages of birds begin and end at optimal times, and do not generally last shorter or longer than the optimal durations. Further, all these stages remain in a defined phase relationship and generally center on reproduction that occurs at the most appropriate time of the year when the food resources in the wild are optimally present and the chances of survival of the young and parents are maximum. Therefore, the timing of actual reproduction is critical for the species. To achieve successful breeding, most birds use environmental factors like photoperiod, rainfall, temperature, vegetation, food availability etc. to up- and down- regulate the hypothalamic-pituitary-gonadal (HPG) axis. The best studied of such environmental cues is photoperiod (proximate
factor and photic cue) which is considered to be an ‘initial predictive cue’ to initiate reproductive development. In some species, photoperiod may be sufficient to induce complete reproductive development. But in majority of species, additional cues (‘supplementary cues’ and ‘synchronizing and integrating cues’ or ultimate factors and non-photic cues) such as food availability, rainfall, temperature or social interactions are used to fine-tune the timing of completion of reproductive development and onset of breeding. They help decide when the actual event would occur within a particular time window. The degree to which these factors affect the physiology of breeding depends on the species as well as the environment considered. While photoperiod plays an important role in seasonal timing, it cannot account for the annual variation in reproduction. Also, for most species, the increase in biomass of their food resource is temperature dependent. The solar cycle causes extreme changes of light intensity and wavelength in the environment from day to night, and many birds have become specialised for locomotor activity at a particular phase of the 24 h day. Because the light environment varies both in terms of intensity and spectrum, it is logical to expect that endogenous clocks regulating daily and seasonal responses will be sensitive to both the light intensity and light spectrum, besides duration.

It has been suggested that the response system, in most bird species, must have a circadian component related to day length. The circadian rhythm of photoperiodic photosensitivity (CRPP) mediates photoperiodic regulation of reproductive responses in some birds. CRPP responds to light in a phase dependent manner. Bünning proposed the involvement of an endogenous daily rhythm in the photoperiodic response mechanism. This hypothesis involves a circadian (i.e. of about 24 h in duration) component of sensitivity to daily light within the organism as a physiological basis of photoperiodism. According to Bünning, the endogenous circadian rhythm consists of two halves. The first
twelve hour is the subjective day or photoinsensitive phase while the latter twelve hour is the subjective night or photosensitive phase. The photogonadal stimulation is the result of direct or indirect, repeated (daily or otherwise) illumination of the photosensitive phase by external photophase. This device functions as a clock to measure the day length. This model attributes a dual role to light, i.e. entrainer and inducer.

Birds are exposed to day-to-day variation in light hours, to which they adapt using their endogenous clocks because of their great precision in the timing of various behavioural and physiological events. Timing of reproduction and other seasonal events in captivity approximate their timing in the wild indicating the presence of internal timing program. These systems are innate (endogenous), inheritable and genetic in origin. These endogenous clocks can also be important in timing life-history events, but these are generally set by exposure to exogenous factors, e.g. photoperiod as a zeitgeber. These oscillatory systems are synchronized with day and night, and thus, are expressed as daily overt rhythms in the natural environment.

Birds are immensely valuable study systems because they show a dramatic seasonal reorganization in physiology, morphology and behaviour. Further, since birds have excellent clock system, they can be the excellent model of research to understand the biological timing in vertebrates. They show well defined circadian rhythmicity. They are unique among vertebrates having distinct physiological and behavioural features that change with the change of season. Male and female birds differ in many aspects of reproduction in relation to physiology, morphology and behaviour, however, very little is known about possible sex differences in response to environmental cues that control the timing of seasonal breeding. An extensive study to investigate their roles in the regulation of seasonal responses in female birds is scanty or rather lacking. Synchronizing male and female reproductive development within pairs is prerequisite for
a bird to breed successfully. Therefore, it is considerably reasonable to perform serial and thorough experiments involving photic and non-photic cues on both the sexes in order to reveal the mysteries of environmental control of reproduction in birds.

The North-East region offers significantly different environmental conditions compared to the other parts of India, and this could be advantageous in making comparative studies and to enable us to address evolutionary aspects of regulation of seasonal cycles in birds. Also, less is known about the importance of day length, light wavelength and light intensity and associated factors in control of reproduction of seasonal breeders at low latitudes, e.g. in tropics and subtropics. The fact that birds inhabiting both high- and low- latitudes can discriminate even small changes in photoperiod, light wavelength and light intensity reveals that they necessarily represent adaptations by inhabiting different photoperiodic environments. Thus, a study on photoperiodic adaptations on the species that occupies temperate as well as tropical/subtropical photoperiodic environments could be more interesting.

This thesis centers on the photoperiodic investigations carried out on both the sexes of tree sparrow in the subtropical region at Shillong, India (Latitude 25°34´N, Longitude 91°53´E). Much emphasis is particularly laid on studying seasonal and circadian responses (reproduction and associated events) of the tree sparrow in different wavelengths and intensities of light under different artificial photoperiods in both long and short term experiments. We have also attempted to assess the modulatory role of temperature in photoperiodic regulation of reproduction and associated events. The questions on how photoperiodic responses and various characteristics of circadian locomotor rhythms are modulated under the influence of different wavelengths and intensities of visible light have also been addressed through carefully designed experiments. The experiments included in this thesis are focussed on the following main
objectives: (i) to investigate the involvement of photoperiodic time measurement during the induction of gonadal growth and functions (ii) to study the effects of different wavelengths and intensities of light on gonadal growth, bill colour, feathers moult and body weight (iii) to study the entraining- and inducing- properties of different wavelengths and intensities of light in photoperiodic responses and (iv) to study the effects of different temperatures on photoperiodic responses. These are described under a study heading, and finally incorporated into following broader chapter headings:

Chapter I: Photoperiodic time measurement and photoentrainment of circadian activity rhythm

In this chapter, we tried to determine the nature of mechanism(s) involved in the photoperiodic time measurement in both the sexes of tree sparrow. Investigations on photoresponses of the tree sparrow have revealed the photoperiodic nature of this bird. In this chapter, we made an attempt to find out (i) whether a circadian rhythm is involved in photoperiodic time measurement during the induction of gonadal growth and functions and (ii) whether illuminations of different parts of the photoinducible phase (Φi) induce varying degrees of gonadal and circadian responses using skeleton photoperiodic schedules. It was found that the bird responds to some photoperiods and not to all and also its photoperiodic responses vary with the change in photoperiod suggesting that its photoperiodic response system has an efficiency to measure photoperiodic time with a considerable degree of accuracy. Detailed experiments were, therefore, undertaken to resolve the question of the mechanism of photoperiodic time measurement using a novel experimental design, the night interruption experiment.

Experiment 1. Night interruption experiment

This experiment was performed to investigate whether a circadian rhythm is involved in photoperiodic time measurement during induction of gonadal growth and
hormones. Photosensitive birds of both sexes 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. Observations on the gonadal sizes revealed significant gonadal growth in all the birds of G1, G2, G3, G4, G5, G6 and G7 (the birds of these groups behaved as if they had been exposed to stimulatory photoperiods; long day). On the other hand, no significant response was observed in both the sexes of tree sparrow in G8 and the control group (birds in these groups behaved as if they were under non stimulatory photoperiods; short day). 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. The increase and decrease in serum levels of testosterone and estradiol-17β ran almost parallel to increase and decrease in testicular and follicular size, respectively under stimulatory light regimes. A significant increase in the testosterone and estradiol-17β levels was observed in the birds of groups (G1-G7) when compared to C or G8. However, clear sexual differences in the attainment of the peak gonadal size and steroid levels were noticed that occurred in G4 in males and in G5 in females. Further, gonadal growth rate varied significantly among various light-dark cycles. Gonadal growth rate increased significantly in all cycles except G8 in males and G1 and G8 in females when compared with the control (7L/17D). The results can be interpreted on the basis of the Büning hypothesis and external coincidence model according to which the photoperiodic response in sparrows is the result of the coincidence of light with the photoinducible phase of an entrained endogenous circadian rhythm. These results clearly indicated that tree sparrows possess a photoperiodic response system that can detect changes in photoperiod involving endogenous circadian rhythm to time their reproductive functions.
They indicate the involvement of endogenous circadian rhythm in photoperiodic time measurement during induction of gonadal growth and function. The position of the light pulse relative to the photoinducible phase of circadian rhythm of photoperiodic photosensitivity (CRPP) determines a photoperiodic gonadal response in the tree sparrow. The photoinduction occurs, if and only when, light coincides with the photoinducible phase while light falling in the non photoinducible phase produces a short day effect.

**Experiment 2. Investigation on locomotor activity**

This experiment was done to investigate whether illuminations of different parts of photoinducible phase (Φi) causes varying degrees of circadian responses. 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 each. Birds, when exposed to a light-dark cycle of 12L/12D, showed entrainment of their activity rhythm with activity confined mainly in the light phase. A significant variation in the circadian activity was noticed under various night interruption light-dark cycles. The activity movements per day were found to be significantly 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. Furthermore, the mean activity per hour for 24 h differed significantly among various light-dark cycles. It increased gradually with the increase in time of 1 h light interruption of the 17 h dark
phase until it reached a peak in the cycle of 6L/10D/1L/7D and declined gradually thereafter to reach the minimum value under 6L/17D/1L/0D. The analysis, using the cosiner regression curve based on the activity movement, revealed sinusoidal rhythmicity in all the night interruption cycles. There was a gradual increase followed by a decrease in \( \alpha/\rho \) with the increase in time of 1 h light interruption of the 17 h dark phase. Sparrows display circadian rhythm in their locomotor activity that can be entrained to 24 h complete and skeleton light-dark cycles. Birds exhibit variations in various circadian characteristics under different night interruption cycles suggesting that the photoinducible phase is differentially sensitive to light.

Chapter II: Effects of different wavelengths of light on circadian and seasonal responses

This chapter includes experiments that were performed on tree sparrows to investigate wavelength dependent effect on photoperiodic gonadal and behavioural responses and to compare these seasonal responses with those of their conspecifics and other related species in the subtropical and temperate zones. This chapter was divided into following three experiments: (i) Effects of different wavelengths of light on seasonal responses (ii) Entraining and inducing effects of different wavelengths of light on reproductive and related responses and (iii) Effects of different wavelengths of light on circadian characteristics of activity rhythm.

Experiment 2.1. Effects of different wavelengths of light on seasonal responses

This experiment aimed to investigate if the tree sparrows show differential reproductive and associated responses to different light wavelengths and whether the photoreceptors mediating the photoperiodic responses have differential spectral sensitivity. Photosensitive birds of both the sexes were kept under two different photoperiods i.e. 9L/15D (short day length: SD) and 14L/10D (long day length: LD) for
8 months (240 days). In each light regimes, they (n=8 each sex/wavelength) experienced three different light wavelengths: red (long wavelength: 640 nm), green (short wavelength: 500 nm) and white (400-700 nm) at the same irradiance of 14.175 w/m². Periodic observations on testicular and follicular size, bill colour and body weight were made at monthly intervals, while moult of body feathers and wing primaries was recorded fortnightly. In addition, the above observations were also made at the beginning and end of the experiment. Results obtained from the present experiment clearly suggest that different wavelengths of light influence photoperiodic response system differentially in the regulation of seasonal responses in the tree sparrow. The tree sparrows showed a gradual increase in gonadal responses to LD cycle of various wavelengths with the same intensity, while no response was evident under SD of red, green and white light with the same intensity. The gonadal responses to LD also differed significantly in different wavelengths, with the red light giving maximum and earlier (60 days) response followed by white and green wavelengths. They exhibited gradual darkening of bill colour with the increase in gonadal size, attaining darkest colour at peak gonadal size, followed by its lightening during gonadal regression in all the wavelengths of light under LD. Thus, the changes in bill colour ran almost parallel to changes in gonadal size suggesting its control by gonadal steroids from photostimulated gonads. Moult in the body feathers and wing primaries progressed with gonadal regression in tree sparrow. Our data showed that in long day lengths of different wavelengths, the body feathers moult, in both sexes, started with gonadal regression from day 90 reaching to a maximum when observed on day 195 under white light, while in green and red light, there was initiation of moulting after the completion of gonadal regression and the moulting was incomplete. Thus, tree sparrows exhibited a delayed and incomplete moulting cycle of their body feathers under red and green lights while it was faster and complete in the white light. Almost similar
pattern was observed in the moult of primary feathers. On the other hand, birds failed to undergo feathers replacement under non gonadostimulatory photoperiod (9L/15D) of any wavelength, suggesting that longer duration (above threshold photoperiod: between 10-11 h) of photostimulation is required to induce moult. Feather replacement progressed with gonadal regression in tree sparrow suggesting that the two high energy demanding processes in the life-history i.e. breeding and feather replacement occurred at two different phases in annual cycles of the bird. No significant change in the body weight was observed in all the three light wavelengths under both light regimes during the course of the experiment. The tree sparrow uses photoperiod to regulate its seasonal responses. However, apart from the duration of daily light, the light wavelength also helps to determine the light energy required by the circadian processes mediating seasonal responses. Our findings suggest that the physiological processes involved in photostimulation and photorefractoriness in the tree sparrow are sensitive to changes in light wavelengths as they are to changes in day length. Thus, the tree sparrows are most photoresponsive to wavelengths at the far end of the visible spectrum which stimulates the reproductive axis much stronger and faster than the wavelengths at the near end, but the effect, photostimulation or photoinhibition, is dependent on the duration of the light period.

**Experiment 2.2. Entraining and inducing effects of different wavelengths of light on reproductive and related responses**

This experiment was designed to examine the relative importance of different wavelengths of entraining and inducing pulses in a skeleton photoperiodic schedule (SKP) in the regulation of reproductive and related responses in the tree sparrow. For this, red, green and white lights were used in same or different combinations as entraining and inducing pulses in relation to an endogenous circadian rhythm of
photosensitivity mediating photoperiodic responses. An attempt was also made to find out which wavelength of the visible spectrum (out of red and green) is more effective and/or ineffective in inducing a photoperiodic response when used as an entraining and/or inducing light in SKP. Photosensitive birds were exposed to skeleton light-dark schedule (6 hours light: 6 hours dark:1 hour light:11 hours dark; 6L:6D:1L:11D) of 24 h cycle with 6 h entraining and 1 h inducing light of different wavelengths (red, green and white) at equal irradiances of 14.175 w/m². The entraining and inducing light pulses were either of the same wavelengths out of green (G), red (R) or white (W) such as R/R, G/G and W/W (control) or of two different wavelengths in various combinations, e.g. green as entraining light and red as inducing light in one group and vice-versa in the other group in the following combinations: R/W; W/R; G/R; R/G; G/W; W/G. The experiment ran for a period of eight weeks. Tree sparrow exhibited gonadal growth when red or white wavelengths were used either as both entraining as well as inducing pulses or when one was used as entraining and the other was used as inducing wavelength and vice-versa (i.e. R/R, W/W, W/R and R/W). However, gonadal growth failed to occur when the green light was used as entraining as well as inducing pulse or it was used in combination with red or white light (G/G, G/R, R/G, G/W and W/G). Highest gonadal responses, in both the sexes, were noticed when birds were exposed to red light as entraining and inducing pulses (i.e. R/R) followed by entraining and inducing pulses in the wavelength combinations of W/R, R/W and W/W. It was observed that the photoperiodic induction was faster and greater in the group that received red light than those that received white or green light. Tree sparrows receiving both entraining and inducing pulses of white light showed an intermediate response, greater than green but lesser than red light. This trend of photoperiodic induction (red>white>green) in tree sparrow, under skeleton spectral LD cycles, resembled to that found under complete
spectral LD cycles. Our results obtained in this experiment further confirmed that the photoperiodic clock in tree sparrow is differentially responsive to different light wavelengths (at equal irradiance) during its various circadian phases. Also, the response of tree sparrow differs when wavelengths of morning and evening light pulses in SKP were exchanged. This is an indication that different phases of the photoperiodic oscillator are differentially sensitive to light wavelengths. The response of photoperiodic clock to light wavelength in a phase-dependent manner may have adaptive implications. Although the light environment is always dominated by red light, there are very precise spectral changes during the day, especially during twilight periods. It is advantageous for a species, especially a long day breeder like tree sparrow, to be equipped with a circadian clock, which is responsive to the spectral changes of the light environment.

**Experiment 2.3. Effects of different wavelengths of light on circadian characteristics of activity rhythm**

The present experiment was planned to investigate further the effects of different wavelength of light on changes in various circadian characteristics using skeleton photoschedule. In this experiment, we have investigated the influence of three different wavelengths of light (red, green and white) on circadian locomotor activity of tree sparrow maintaining equal energy level. Adult birds when subjected to 12L/12D light conditions for 14 days showed entrainment of their locomotor activity to this light regime, with their movements confined mainly in the light phase. The cosiner regression curve of circadian activity in tree sparrows exhibited a distinct sinusoidal rhythmicity with a period of 24 h. When these birds were transferred to night interruption schedules of 6L/6D/1L/11D with entraining and inducing pulses of different wavelengths of light, their activity splitted mainly between 6 h of entraining and 1 h of inducing lights. Intra group comparisons of the activity movements per hour at intervals of 14 days for the
treatments 15-42 days (comprising white light as entrainer and inducer throughout in control group; white light as entrainer and inducer for first 14 days followed by red as entrainer and green light as inducer for another 14 days and then the green as entrainer and red light as inducer for last 14 days in group one; white light as entrainer and inducer for first 14 days followed by green as entrainer and red light as inducer for another 14 days and then the red as entrainer and green light as inducer for last 14 days in group two) revealed no significant difference in activity movement per hour. In group one (G1), the activity was recorded highest in 6W/6D/1W/11D (P<0.05) which was followed by 6R/6D/1G/11D (P<0.01) and lowest in 6G/6D/1R/11D. However, group two (G2) birds behaved differently showing no significant changes in activity movement in various combinations of light wavelengths. Further, inter group comparisons for the treatments from 29 to 42 days showed that the activity movement per hour was highest in the birds of G1 that were exposed to red light as entrainer and green as inducer (6R/6D/1G/11D), intermediate in the birds of G2 exposed to green light as entrainer and red as inducer (G2: 6G/6D/1R/11D) and least in the birds of G3 exposed to white light both as entrainer and inducer (G3:6W/6D/1W/11D). However, the interchange of red and green wavelengths of light as entraining and inducing in the treatment from 43 to 56 days in G1 and G2 did not affect activity movement per day in our study birds. The analyses of data involving intra and inter group comparisons of activity movement per day during various treatments from 15 to 56 days revealed almost similar results as obtained in case of activity movements per hour above.

When the groups were finally transferred to LL_{dim} for another 14 days, the birds showed their endogenous rhythmicity and exhibited free-running condition with a phase advance. The birds exposed to these cycles exhibited significant changes in various circadian characteristics. They showed significant variations in activity period (\(\alpha\),
resting period (\(\rho\)) and ratio of \(\alpha/\rho\). Further, significant group differences in activity onset were noticed when we compared the start of the activity with lights ON and the end of activity with the lights OFF. Thus, birds of all the groups showed early onset of activity in 6W/6D/1W/11D followed by 6R/6D/1G/11D and 6G/6D/1R/11D while the activity offset occurred later in 6G/6D/1R/11D which was followed by 6R/6D/1G/11D and 6W/6D/1W/11D.

Thus, it is clear from the present experiment that the circadian rhythm of locomotor activity in sparrow can be entrained to 24 h complete and skeleton light-dark cycles. Birds show continuous activity during the light hours and the activity subsides at lights off. The tau of activity-rest rhythm, as calculated from the activity data of 14 days in the free running condition, did not show any significant difference in the birds of various groups experiencing treatments involving changes in wavelengths and light regimes. Thus, we may conclude from the above findings that tree sparrow exhibit almost similar pattern of activity behaviour under various light regimes. However, there are significant differences in characteristics of circadian activity rhythm under different wavelengths of entraining and inducing light provided at equal irradiance.

**Chapter III: Effects of different intensities of light on circadian and seasonal responses**

This chapter includes investigations on the influence of light intensity on photoperiodic seasonal and daily responses. In particular, the emphasis was placed on studying seasonal and circadian responses of captive birds in three different intensities of light under long and short day lengths and night interruption schedules. The experiments conducted under this chapter are summarized in the following two experiments: (i) *Effects of different intensities of light on seasonal responses* (ii) *Influence of different*
light intensities on the entraining and inducing properties of light in gonadal and body weight responses.

**Experiment 3.1. Effects of different intensities of light on seasonal responses**

In this experiment, we investigated the interactions of duration (long- and short-photoperiods) and three different light intensities on the photoperiodic regulation of gonadal growth and regression together with other associated physiological changes reflected in bill colour, moult of primaries and body feathers and body weight. This experiment was done to test whether the birds’ photoperiodic response system is influenced by different light intensities. Photosensitive birds of both the sexes were subjected to two different photoperiods, i.e. 9L/15D and 14L/10D for 8 months (240 days). In each light regimes, they were divided into three groups and exposed to three different light intensities: 50, 400 and 1000 lux. Periodic observations on testicular and follicular size, bill colour and body weight were made at monthly intervals, while moult of body feathers and wing primaries was recorded fortnightly. In addition, the above observations were also made at the beginning and end of the experiment. Significant gonadal response was found in all the three intensities of light under long day length in both the sexes but not in the short day length. In males, the testicular volume started increasing gradually by day 30 in all the three intensities of light, reaching to its peak when observed on day 60 in 400 lux, however, attainment of peak testicular growth was delayed for a month, i.e. day 90 in 50 and 1000 lux after which birds in all groups underwent gonadal regression. Similarly, in females, follicular diameter started increasing gradually by day 30 in all the three intensities of light, reaching to its peak on day 60 in 50 and 400 lux, while it was delayed for a month (i.e. day 90) only in 1000 lux. Follicular regression was evident in all the groups thereafter. The changes in bill colour corresponded to that of the gonadal growth. Birds of both the sexes exposed to a
stimulatory light regime of 14L/10D showed significant moult in their body and primary feathers in all intensities of light. Maximum and complete moult in both types of feathers was observed in 400 lux. Though the mouls were evident in 50 and 1000 lux, they were found delayed and incomplete. On the other hand, birds failed to undergo moult in both types of feathers in all intensities of light under non gonadostimulatory photoperiod, i.e. 9L/15D. Birds maintained their normal body weight throughout the experiment with some statistically insignificant variations. The intensity of light, therefore, proved to have differential effects on the photoperiodic seasonal responses in both the sexes. Our results, thus, demonstrate the differential role of light intensity in fine tuning photoperiodic seasonal responses in the tree sparrow and suggest that its photoperiodic response system is capable of discriminating different light intensities.

**Experiment 3.2. Influence of different light intensities on the entraining and inducing properties of light in gonadal and body weight responses**

This experiment was done to investigate the entraining and inducing properties of different light intensities in the circadian rhythm of photoperiodic photosensitivity (CRPP) in photoperiodic regulation of reproductive and related responses. An attempt was also made to examine if interchange of light intensities between first (morning) and second (evening) light pulses could also influence photoperiod-induced gonadal response in the tree sparrows. In this experiment, we have, therefore, investigated the phase-dependent effects of different light intensities on the photoperiodic clock of the tree sparrow. Photosensitive birds were exposed to skeleton light-dark schedule i.e. 6L/6D/1L/11D with different combinations of entraining and inducing light intensities, such as 6L^{25}lux/6D/1L^{200}lux/11D, 6L^{200}lux/6D/1L^{25}lux/11D and 6L^{400}lux/6D/1L^{400}lux/11D for two months. Periodic observations on gonadal size, in both the sexes, showed significant and highest gonadal response when 400 lux was used as both entraining and inducing
pulse, followed by that of $6L^{25\text{lux}}/6D/1L^{200\text{lux}}/11D$ combination and the least response was evident in $6L^{200\text{lux}}/6D/1L^{25\text{lux}}/11D$ combination. Further, when 25 and 200 lux of light intensities were exchanged for their roles as entraining and inducing agents, gonadal growth was found to be significantly higher when 200 lux was used as inducing agent. No significant change in the body weight of the birds was obtained under any treatments and they maintained their normal body weight throughout the experiment. This study, thus, revealed that the low- and high- light intensities have their differential effects in CRPP in inducing photoperiodic gonadal responses. A higher intensity of light is more effective in inducing gonadal response than the lower intensity. Thus, higher the intensity of inducing light above the threshold, higher is the photoperiodic gonadal response in the tree sparrow. This may have adaptive implications for a subtropical species.

Chapter IV: Effects of different temperature conditions on photoperiodic seasonal responses

This chapter was planned to investigate the modulatory role of temperature on the photoperiodic regulation of seasonal reproduction and related functions using both sexes of tree sparrow. Also, to test if confinement to captivity and/or exposure to artificial temperature condition will affect the temporal phasing of the seasonal cycles. In the first experiment, birds were exposed to two different conditions: (i) the captive birds receiving natural light and temperature conditions and (ii) captive birds receiving natural light and a constant temperature of $\sim17\pm2^\circ\text{C}$. The seasonal cycles of gonads, bill colour, feathers moult and body weight were recorded. Our study on the tree sparrow showed the modulatory role of temperature on the photoperiodic control of reproduction and moult in the tree sparrow. Birds of both the sexes held under both natural and constant temperature conditions exhibited gonadal growth followed by regression and
development of photorefractoriness. Although no significant difference in either gonadal or bill colour response was noticed in the birds under the above two conditions, there were significant differences in the primary and body feathers moult. The moult in both the feathers was found delayed in constant temperature condition when compared to birds under natural condition. No significant change in body weight was observed in either sex under both the treatments.

In the second experiment, tree sparrows were exposed to artificial long (14L/10D) and short day (9L/15D) lengths. In each light regimes, groups of birds were subjected to three different temperature conditions viz. 17°C, 25°C and 30°C. Observations on the gonadal size, bill colour, body and primary feathers moult revealed significant differences in all the three temperature conditions under long day photoperiod. On the other hand, birds of both the sexes showed gonadal growth, darkening of bill colour and feathers moult only at 30°C under short days. However, the responses at 30°C under short days were significantly lesser as compared to the responses at 17°C, 25°C and 30°C under long days. There were significant variations in the photoperiodic responses among the birds maintained at different temperatures under long days. More or less similar pattern was followed in bill colour response that ran almost parallel to changes in gonadal size. Further, the active period of gonadal function was found to be longer in the birds at 17°C when compared to birds at 30°C. The rate of gonadal regression in our study bird was found to be slower at the lower temperature of 17°C when compared to higher temperatures of 25 and 30°C. Both sexes exhibited moult in their primary and body feathers at all the three temperature conditions under long day length. An early and complete moult was observed in the birds maintained at higher temperature of 30°C while it was delayed in the birds at 17 and 25°C. Tree sparrows maintained under short day length showed feathers moult only at 30°C, a temperature
condition that also induced gonadal development indicating that the two life-history stages of reproduction and moult are somehow related in this species. These results clearly indicate that although there is little effect of temperature on gonadal maturation in tree sparrow, the gonadal growth and regression is delayed under lower temperature. Sparrows did not show cyclicity in their body weight under any of the above three conditions and the birds maintained their normal body weight throughout the experiment.

From the present study, we can conclude that the subtropical population of tree sparrow is photosensitive and is able to discriminate the changes in daily photoperiods as well as to changes in wavelength and intensity of light. It emphasizes the importance of photic (duration, wavelength and intensity) cues as significant environmental variables in the control of seasonal and circadian responses (reproductive and behavioural) in both the sexes. It also reveals the role of a non-photic cue (i.e. temperature) in fine-tuning the reproductive and associated seasonal events. Thus, while the photic cues control the seasonal reproductive and associated responses in the tree sparrow, the non-photic cue might help in their fine-tuning. This thesis, thus, contributes significantly further for a better understanding of the role of day length as it focuses on the differential roles of three important characteristics of daily light i.e. duration, wavelength and intensity in control of seasonal reproduction and associated functions in the birds inhabiting subtropical regions. On the basis of the above findings, it may be concluded that despite of little changes, the tropical and subtropical birds are also able to discriminate light in its different characteristics in control of their seasonal and circadian responses like their temperate counterparts. This thesis, therefore, adds significant contributions to the field of avian photoperiodism as it comprises of investigations carried out on both the sexes of tree sparrow in the subtropics where the information on photoperiodic control of avian seasonality is scanty. They play a significant role in regulation and modulation of
reproductive and associated events in the birds’ annual cycles (i.e. both seasonal and circadian). However, further studies aimed to understand the mechanism(s) regarding perception of different colours and intensities of light are required to understand the complex avian photoperiodic response mechanism. In addition, it is the need of the hour to investigate the mechanism by which temperature affects the avian reproductive and associated responses.