Photoperiodic regulation of seasonality in reproduction in the yellow-breasted bunting, *Emberiza aureola* (Pallas)

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Abstract

Among vertebrates, birds exhibit remarkable seasonality in several functions, such as seasonal cycles in gonadal growth and development, body fattening, migration, moult, food intake, song production, hormone secretion etc. A seasonal cycle, represented by the initiation, termination and reinitiation of physiological processes, is a compulsory adaptation that ensures the occurrence of reproductive and associated events 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 youngs and parents are maximum. Further, predictability of seasonal change is important because, in order to match their behaviour to the seasons, birds need to predict the optimal time for each life-history stage to complete the necessary changes in physiology in advance. Birds occupy habitats which are different in amplitude and the predictability of environmental factors. They also differ in the geographical ranges they inhabit over the course of their lives, resulting in the difference in timing and sequences of life-cycle stages. These stages in a species remain in a distinctive phase relationship and generally centre on reproduction. Therefore, the timing of actual reproduction is critical for the species. Birds having annually periodic reproductive season may use periodic changes in the environment as predictive information for the oncoming of favourable season for reproduction. The time and duration of the favourable season selected for reproduction differ amongst different climatic regions and different ecological groups of birds. Several environmental factors help in this timing, and important ones include seasonal variations in day length, temperature, rainfall, humidity and vegetation etc.

Of several factors in the environment, which the birds are exposed to, the day length is most consistent and reliable. Day length changes with the season, at least away from the equator and the intensity of light at any one time of day changes with the weather even at the equator. 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. It acts as a proximate factor providing information about yearly variation in seasons and predicting the time of favourable conditions when offspring are most likely to survive. A wide variety of birds, therefore, use the day length or photoperiod as an anticipatory cue to make seasonal preparations. There are reports that the long daily photoperiods induce gonadal growth followed by regression while short daily photoperiods fail to do so in a number of temperate and subtropical birds. The non photic cues like food, temperature, rainfall etc. act possibly as ultimate factors or supplemental cues that help decide when precisely the actual event would happen in a given time window in a variety of avian species. The degree to which non photic factors affect the physiology of breeding depends on the species as well as the environment considered.

Timing of reproduction and other seasonal events in captivity approximate their timing in the wild indicating the presence of internal timing program. Birds tend to adapt to daily light-dark cycle using their endogenous time-keeping device(s), called "clocks" because of their great precision in the timing of various behavioural and physiological events. This endogenous program enables the birds to identify the time when to switch on (photoinduction) and when to switch off (photorefractoriness) their physiological mechanisms so that the seasonal events occur at the most suitable time of the year. In photoperiodic birds, a circadian rhythm of photoperiodic photosensitivity (CRPP) mediates photoperiodic regulation of reproductive responses. CRPP responds to light in a phase dependent manner. Such a concept was originally formulated by Bunning and involves the operation of an external coincidence model. It predicts that photoperiodic induction occurs when the light coincides with the photosensitive phase or more precisely photoinducible phase of an entrained endogenous circadian rhythm, which occurs early in subjective night. This model attributes a dual role to light, i.e., entrainer and inducer. Thus, the coincidence of light with the period of maximum inducibility of the endogenous clock occurring about 12 hours after the sunrise in a long day breeder, as in spring and summer months, is read as “long days” and leads to the photoperiodic induction. Failure of such coincidence, as would be the case during the winter months when daily light is shorter than 12h per day is read as “short day; consequently, there is no photoperiodic induction. Instead the short days tend to mediate the recovery of the photosensitivity in refractory individuals.

Birds being the most highly mobile vertebrate class are extremely well adapted to migration, and the phenomenon is displayed on a large scale within this group. Most typically, avian migration is seen as a “regular, seasonal, long distance, large-scale shift of the population twice a year between a restricted breeding area and a restricted wintering area”. It has adaptive value as it permits the use of a seasonally favourable region for reproduction. Therefore, in principle, the mechanisms controlling reproduction and migration seem to be similar and coordinated. Migration, moult and reproduction are three energetically exhaustive physiological processes in annual life span of birds. In order to meet the caloric demands of these physiologically important events, majority of species adapt mechanisms, which make these events to escalate well spaced from each other. In some species, migration is completed before gonadal recrudescence begins, although these tend to be short distance migrants. Yet others show varying degrees of gonadal development during migration, indicating that the development phase of breeding can overlap entirely with mature capability of migration, and the termination phase of migration may overlap with mature capability of the breeding stage. Photoperiod seems to regulate various physiological events associated with migratory phenomenon such as development of migratory urge (zugdisposition) which is manifested by fat accumulation, hyperphasia and body weight increase along with *zugunruhe*, the migratory restlessness Photoperiodic control of premigratory fattening has also been observed in a few subtropical migratory visitors in which testicular cycle is photoperiodically controlled. Migratory bird exhibit pronounced seasonality in body mass because heavy fat deposition serves as "fuel" for migration in them. Moult is the periodic replacement of feathers. Most bird species need to completely replace their plumage each year, and so a period for moult needs to be included in the annual cycle. Avian moult replaces worn feathers and is seasonal, with most passerines moulting once or twice each year. Breeding and moult are normally separated because both are energetically demanding. Moult most often starts after breeding but it may occur on the wintering ground after migration in some species. Synchronization of reproduction and moult rhythms by annual photoperiodic cycles is usually achieved through seasonal changes in responsiveness to photoperiod. Further, photostimulation is required to induce moult but it also induces gonadal maturation and regression. Therefore, it is not clear whether photoperiod has a direct effect on moult, or whether it is a secondary consequence of photoperiodic control of the gonadal cycle and a physiological link between gonadal regression and moult, satisfying the ecological requirement for moult to follow breeding.

The role of day length as primary environmental factor regulating reproduction and associated functions in very substantial number of avian species inhabiting mid and high latitudes is relatively well established. Less is known about the importance of day length in controlling these functions in the birds inhabiting/visiting tropical and subtropical regions. Because of small annual variations in the tropics and subtropics, photoperiod has been speculated to be of little use in regulating metabolic and reproductive functions of birds. Interestingly, many low latitude avian species show annual reproductive cycles and photoperiodic responses that often resemble the seasonal breeding strategies of temperate birds. 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. Therefore, critical field and laboratory experiments are required to ascertain the role of photoperiod in regulation of reproduction, moult and migration in the birds with greater attention on migratory species. Moreover, the majority of investigations concerning the effects of photoperiod on avian reproduction and related functions have been limited to studies on males. Males and females differ in relation to many aspects of reproduction related to physiology, morphology and behaviour. Therefore, in order to validate the generalisation regarding the photoperiodic system of a bird species, it is important to investigate female counterparts also on which information is scanty. The fact that birds inhabiting both high and low latitudes can discriminate even small changes in photoperiods reveals that they necessarily represent adaptations by inhabiting different photoperiodic environments. Our knowledge about the regulation of seasonal cycles in the migratory birds visiting India is very limited. The available information is mostly confined to non migratory wild species unlike in Europe, America and Japan where mostly migratory or domestic species have been studied. This thesis includes investigations on both the sexes of yellow-breasted bunting (*Emberiza aureola),* a migratory bird visiting North-east part of India (Shillong: Lat. 25°34´N, Long. 91°53´E), to address the role of photoperiod in regulation of seasonality in reproduction and associated events. An attempt has also been made to assess the modulatory effects of temperature, if any. In particular, the emphasis is placed on studying seasonal responses of the yellow-breasted bunting under natural and artificial photoperiodic conditions in both short and long term experiments. A specific question on how circadian rhythms and photoperiodic induction is possibly modulated has been addressed through carefully designed experiments. The experiments included in this thesis focus mainly on three broad objectives: (i) to investigate seasonal cycles of gonads, gonadal hormones, body weight and fattening, moult, and bill and plumage colour in captive birds maintained under natural and temperature controlled conditions (ii) to determine the role of photoperiod in regulation of seasonal cycles and (iii) to study the involvement of endogenous circadian rhythm(s) in timing photoperiodic responses. These are described under a study heading, and finally incorporated into following broader chapter headings:

Chapter I Seasonal cycles under natural and temperature controlled conditions

This study was accomplished by recording morphological (size of the gonads, body weight and fattening, bill and plumage colour, and moult pattern), histological (testes and ovary) and hormonal (Testosterone and Estradio-17β) changes in the birds of both the sexes held in an open aviary receiving natural light and temperature conditions and in a closed aviary experiencing natural light but a constant temperature of about 18^{o}C at Shillong (25°N) during different months of the year 2009. It revealed that both the sexes of captive buntings possess definite annual cycles of gonadal size, bill and plumage colour, body weight and fattening, serum levels of gonadal steroids, and moult of primary and body feathers under both natural as well as temperature controlled conditions. Further, both the sexes have, to a great extent, almost similar pattern of seasonal cycles under the two conditions with minor variations. There appears a close correspondence between annual gonadal cycle in buntings and annual changes in day length at Shillong raising the possibility of their photoperiodic control. Increase in day length in spring months induced gonadal recrudescence but the gonadal regression was observed when the day length was still longer than the spring months indicating that the birds become absolutely photorefractory in nature. The annual reproductive cycles, in both the sexes, were found to have four distinct phases with single reproductive peak in May viz. preparatory (December-January), progressive (February-March), reproductive (April-May) and regressive (June-November) phases. A comparison of seasonal cycles in the birds of open and temperature controlled aviaries revealed that the testicular and ovarian cycles under the above conditions followed almost similar pattern of growth and regression. The increase and decrease in the serum levels of testosterone and estradiol-17 β ran almost parallel to the increase and decrease in gonadal size in the buntings suggesting the role of gonadal steroids in control of gonads and gonadal cycles in this bird. The pattern of secondary sexual characters such as annual changes in bill and plumage colours followed the development of gonads and was almost similar in both the sexes under both the conditions. Seasonal changes in the bill colour ran almost parallel to gonadal cycles and also to the serum levels of gonadal steroids suggesting the possibility of its regulation by gonadal steroids. Further, the birds exhibited a significant seasonal cycle in their breast and head colours in both the sexes. Histomorphometric studies of the gonads of buntings revealed seasonal changes in various parameters like gamatogenesis, thickness of germinal and tubular walls, diameters of interstitial and seminiferous tubule in the testes and in the thickness of follicular wall and number of small, large, medium and atretic follicles in the ovary.

Buntings exhibited seasonal cycles in their body weight and fattening that appeared correlated with the annual gonadal cycle. Further, the annual cycles in fattening and body weight changes ran hand in hand with some variations in both sexes. An increase in body weight and fattening were observed in the captive birds under natural conditions when they were experiencing increasing day length of spring raising the possibility of body weight being photoperiodically regulated. Buntings exhibited a complete post nuptial moult of wing primaries and body feathers changing birds into their basic light yellow plumage. Although moult in the body feathers progressed with the gonadal regression in both the sexes, it began one month in advance in males (June) as compared to females (July) and terminated in November in both the sexes. Thus, the post nuptial moult in the body feathers extended from June/July to October in buntings. The moult in the wing primaries began two months later, i.e., in August/September and extended up to November when the gonads were completely quiescent. These results clearly suggest that the feather moult in buntings is somehow linked with gonadal regression when plasma levels of gonadal steroids also decline showing that the two high energy demanding processes of reproduction and post nuptial feather moult are phased at two different times in the annual cycle of the bird. In addition, buntings also underwent a partial prenuptial moult of body feathers only that occurred during spring months (March-May) and resulted into their bright breeding plumage.

On comparing seasonal cycles of male and female buntings, it was found that both the sexes have to a great extent similar general pattern of seasonal cycles. However, the histology and histomorphometric analysis of gonads reveal that the testis reaches to spermatogenic levels while ovarian follicles do not grow beyond the stage of secondary follicle level with complete absence of yolk in captive conditions. Further, females exhibited a less intense seasonal cycle in their breast and head colour as compared to male birds. Body feathers moult was little delayed in male birds when compared with the females suggesting a sex dependent timing of moult cycle in the bunting. A comparison of various seasonal cycles between the buntings under natural and constant temperature conditions revealed that they run almost parallel to each other with minor variations suggesting that temperature probably has little or no role in regulation of seasonal cycles in this bird.

Chapter II Role of photoperiod in regulation of seasonal responses

There appears a close correspondence between annual reproductive and associated cycles in buntings and annual changes in day length at Shillong raising the possibility of their photoperiodic control. This possibility has been tested in the present chapter that aims to assess the importance of photoperiod as a proximate factor in control of reproduction and associated functions in bunting. In different experiments, we have (i) investigated seasonal cycles under programmed photoperiodic schedules (9L/15D, corresponding to shortest day length; 12L/12D, equinox day length; and 14L/10D, corresponding to longest day length at Shillong), (ii) defined critical day length for testicular and body weight responses and (iii) studied seasonal variations in the sensitivity of the photoperiodic response system to long day length (14L/10D). This chapter includes following three experiments aimed to investigate short and long term responses of birds under a variety of photoperiods.

Experiment 1: Investigation under constant photoperiods

Photosensitive bunting of both the sexes, when subjected to three different artificial day lengths: 9L/15D (close to shortest day length at Shillong), 12L/12D (equinox day length) and 14L/10D (close to longest day length at Shillong) for a duration of 540 days, showed gonadal growth followed by regression and development of absolute photorefractoriness with corresponding changes in the levels of sex hormones (testosterone and estradiol-17 β) only under long daily photoperiods (12L and 14L) but not under short daily photoperiod (9L). Though the birds attained peak gonadal growth at the same time under the stimulatory light regimes the gonadal growth and regression were faster under 14L when compared to 12L. Birds did not show reinitiation of gonadal response after the completion of initial growth regression cycle under the stimulatory photoperiods (14L and 12L) even after their exposure to these photoperiods for 18 months, precluding the possibility of involvement of circannual rhythm in their control. Buntings underwent a partial prenuptial moult of body feathers that coincided with gonadal growth followed by an extensive post nuptial body feather moult that progressed with gonadal regression. In contrast, birds exhibited only postnuptial moult of their wing primaries that occurred later than the body feather moult when the gonads have almost regressed. Body moult followed almost similar pattern under both the gonadostimulatary photoperiods (12L and 14L) but the extent of moult was greatly reduced under 12L as compared to 14L. Further, moult was not observed either in the wing primaries or in the body feathers under non gonadostimulatory photoperiod (9L). In addition, birds failed to undergo moult in their wing primaries in one of the gonadostimulatary photoperiod, i.e., 12L suggesting higher photoperiodic requirement to initiate moult in the wing primaries. Thus, the photoperiodic requirement for moult of wing primaries and body feathers differs in bunting. A significant increase in body weight and fattening followed by reduction, leading to minimal value were observed in buntings maintained under 12L and 14L photoperiods, though they did not remarkably follow gonadal growth and regression. On the other hand, short daily photoperiod of 9h failed to induce weight gain in the birds under investigation. This raises the possibility of body weight being photoperiodically regulated in both the sexes of the bunting. On comparing the responses of the female bunting with those of the males, it was found that both the sexes are photosensitive and show almost similar photoperiodic responses with minor variations. In keeping with other findings regarding the photoperiodic effect, the rate of ovarian development was found to be slower than that of testes.

Experiment 2: Threshold photoperiods for seasonal responses

In this experiment, various combinations of duration of light and dark in 24h cycle with increasing proportion of light period (viz. 9L/15D, 11L/13D, 12L/12D, 14L/10D and 16L/8D) were used to determine minimum day length required for gonadal growth and body weight responses in bunting. The data obtained indicate that daily photoperiod of about 12h is important for inducing gonadal growth and functions in both the sexes of buntings as the birds maintained under daily photoperiod of 11h failed to show gonadal response while those experiencing 12h light per day responded significantly. Thus, buntings seem to have a photoperiodic threshold of about 12h per day for gonadal growth and function. Moreover, the extant of gonadal growth was greater in the birds held on longer photoperiods. The threshold photoperiod for gonadal response could not vary between male and female buntings. The natural time of gonadal growth in the captive birds experiencing natural day length corresponds to the photoperiodic threshold observed under artificial photoperiods in the laboratory. These observations further support our assumption that despite the small annual variation in day length of tropics and subtropics (3h and 15min. at Shillong), the bunting might be using increasing photoperiods of spring as a cue in initiating its reproductive responses in nature. Further, bunting showed body weight response to daily photoperiods of 11h and above/day. The threshold photoperiod for liporegulatory functions and body weight increase was, thus, found to be lower (i.e. about 11h/day) in buntings as compared to critical day length for gonadal response (about 12h/day). This suggests that the threshold photoperiod is not only species specific but also response specific in bunting.

Experiment 3: Seasonal variation in photosensitivity

In this study, we have investigated seasonal variation in the sensitivity of the photoperiodic response system in regulation of gonadal and body weight responses in both the sexes of bunting. An attempt was also made to examine the termination of photorefractoriness in the captive birds under natural conditions. Birds of both the sexes, when transferred from the open aviary receiving natural day length and environmental conditions in the beginning of every month of the year to long days (14L/10D) for 12 weeks, showed gonadal growth only in the birds transferred from December to May. It is evident that both the sexes of the bunting are photosensitive and exhibit post reproductive refractoriness. Seasonal responses in bunting cycle between periods of photosensitivity and photorefractoriness. The results further suggest seasonality in responsiveness of the endogenous response system to long days over the year. It appears that the initiation of gonadal growth and the termination of photorefractoriness leading to recovery of photosensitivity are long day and short day phenomena, respectively in this species. This suggests that both long and short days are important for photoperiodic regulation of seasonal reproduction and metabolic events in this species, although the bird uses them for different purposes. Further, a gradual increase and decrease in photosensitivity of the endogenous response system is evident in both the sexes. The photorefractoriness, observed in the annual gonadal cycle, requires shortening days in winter (November-December) in the nature for its termination so that birds become photosensitive once again to the stimulatory effects of increasing day lengths in spring. Seasonal variations in the photoresponse system were also observed in body weight. Further, male and female birds responded almost in the same fashion.

Chapter III: Photoperiodic time measurement

Attempts were made in this chapter to determine the nature of mechanism(s) involved in the photoperiodic time measurement in both the sexes of buntings. Investigations on photoresponses of the bunting revealed the photoperiodic nature of this bird. It has been found that the bird responds to some photoperiods and not to all and also its response vary with the change in photoperiod. This shows that the photoresponse system of the bunting has an efficiency to measure photoperiod somehow with considerable degree of accuracy. Detailed studies were, therefore, undertaken to resolve the question of photoperiodic time measurement using novel experimental designs, i.e., resonance, night-interruption, and intermittent light experiments.

Experiment 1: Resonance experiment

In this experiment, Nanda-Hamner experimental protocol was used to investigate the mechanism of photoperiodic time measurement in regulation of reproduction and also to examine whether the photoperiodic clock(s) in bunting possess a circadian component. Male photosensitive birds were held under different resonance light cycles such as 12- (6L/6D), 24- (6L/18D), 36- (6L/30D), 48- (6L/42D), 60- (6L/54D) and 72-(6L/66D) h along with a control group under long day length (14L/10D). Gonadal growth was observed in the birds submitted to the resonance cycles of 12, 36, and 60h and in control group, while no response was evident in the cycles of 24, 48 and 72h. These results clearly indicated that bunting possess a photoperiodic response system that can detect changes in photoperiod involving endogenous circadian rhythm to time their reproductive functions. They can be interpreted on the basis of the

Bunning hypothesis and external coincidence model according to which the photoperiodic response in bunting is the result of coincidence of light with the photoinducible phase of an entrained endogenous circadian rhythm.

Experiment 2: Night-interruption experiment

Night-interruption light dark (LD) cycles were employed to test the involvement of circadian rhythm in photoperiodic time measurement and to define the gonadostimulatory photoinducible phase in this bird. Birds of both the sexes were subjected to different night-interruption light dark cycles: 6L/6D/1L/11D, 6L/7D/1L/10D, 6L/8D/1L/9D, 6L/10D/1L/7D, 6L/12D/1L/5D, 6L/14D/1L/3D, 6L/16D/1L/1D along with a control group under 7L/17D. Birds in all the night-interruption cycles, except under 6L/14D/1L/3D, 6L/16D/1L/1D and control group 7L/17D, showed gonadal response. This clearly suggests the importance of position of light in endogenous circadian rhythm of photosensitivity in inducing a photoperiodic response. The position of the administered light pulse relative to photoinducible phase of the CRPP determines a response and a photoinduction occurs if and only when light coincides with the photoinducible phase. Further, it was evident from the observations that the rate of gonadal growth increases with increase in time of interruption of the photoinducible phase (ϕi) reaching to maximum at Zt15. Thereafter, it declines and attains minimum levels at Zt21 and Zt23. This indicates differential photosensitivity of the photoresponse system of bunting in the different portions of ϕi. The photoinducible phase in this species seems to be of about 7h extending from about 13-19h after dawn.

Experiment 3: Intermittent light experiment

This experiment was designed to test whether multiple light flashes given during the photosensitive phase are more effective than a single broad light pulse of same or greater duration or whether the gonadal growth depends upon the total amount of light during photosensitive phase. Results obtained from this experiment show that bunting possesses a time measuring system that utilizes an endogenous circadian rhythmicity for reproductive function. They further suggest that multiple flashes of light when given during the photosensitive phase were more effective in inducing gonadal growth and serum levels of testosterone than a single broad light pulse of same duration. Though, all the birds in different intermittent light dark cycles were provided with same length of light i.e., 12h, the testicular growth and testosterone levels were different among the groups depending on the numbers of pulses of light coincident with the photoinducible phase. A decreasing trend of testicular growth and testosterone levels were observed in bunting held under 2L/2D, 4L/4D and 6L/6D receiving 3 light pulses in 2L/2D and 1 in 4L/4D and 6L/6D in the photoinducible phase. This suggests that the photoperiodic response decreases with the decrease in number of light pulse of same duration in the photosensitive phase. Birds under 4L/4D, 6L/6D and12L/12D light regimes showed almost similar response as they received only one pulse of light in the photoinducible phase. Buntings showed testicular response under 12L/12D as the threshold photoperiod for this response is about 12h per day.

It may be concluded on the basis of the present study that the yellow-breasted bunting is a photosensitive species and is able to discriminate the changes in daily photoperiods as small as those normally experienced at Shillong. The bird continues to utilize seasonal changes in day length as a proximate environmental cue to time its seasonal responses even at relatively low latitude (25°N). Its photoperiodic responses resemble to those of closely related species living at lower and higher latitudes despite of significantly different environmental conditions at 25°N, 91°E (Shillong). The present study on both the sexes of bunting reveals the conservation of photoperiodic control mechanisms in them as an adaptive strategy in temporal environment that ensures seasonal events to occur at the most suitable time of the year. Thus, suggesting that despite small annual photofluctuation (3h and 15min. in Shillong), bunting possibly utilise the seasonal changes in day length as a potential time cue controlling their reproductive and associated functions.

The present study emphasizes the importance of day length as a significant environmental variable in the control of seasonal reproductive and associated events in both the sexes of the bunting. The investigations have helped in refuting the notion that the little photofluctuation at subtropics may not be an effective environmental signal for reproductive and associated events. The results obtained from the various experiments of the present study suggest that the photoperiod has more pronounced role in the control of reproduction and/or metabolic functions in the low latitudes inhabiting/visiting avian species as well, than has hitherto been assumed. The thesis, thus, contributes significantly further for a better understanding of the role of day length in seasonal reproduction and associated functions in the birds inhabiting/visiting subtropical regions. This dissertation, thus, adds significant contributions to the field of avian photoperiodism especially to the understanding of biochronometric aspect of photoperiodic control mechanism in birds.