1.1. Organisms Adapt to Environmental Changes

Darwin 1859 suggested that evolution favors the selection of organismal modifications that enhance individual survival of organisms by ensuring their adaptation to environmental conditions in most suitable manner. These adaptations may be in space or time. The spatial adaptations are generally long term changes that appear as structural i.e. morphological and anatomical changes in organisms. Temporal adaptations however may be long or short term, as the organism experiences (1) repetitive changes caused by either earths revolution around the sun or by earths rotation on its own axis; the moons revolution around earth is also a factor that affects periodicity in the life of many organisms. Organisms depend on one or multiple geophysical phenomena; they cope with rhythmic changes in a way that they are able to use opportune range of environmental conditions besides being able to avoid the unfavorable environmental conditions.

The earth rotates on its own axis approximately every 24h, and revolves around sun in 365.25 days. This exposes organisms inhabiting the planet earth to daily and seasonal environmental cycles of light dark, high-low temperature etc. The strict regimen of cyclical changes on planet Earth, envisages that organisms manifest temporal adaptive strategies that help them to anticipate the appropriate season. Most organisms have evolved a mechanism to cope with the temporal programs (daily and seasonal periodicities) so as to adjust their behavioral and
physiological activities in anticipation of the daily/seasonal environmental changes. These adaptive strategies known as biological rhythms involve temporal organization of various endocrine and metabolic activities within the organisms. They are reflected in the widespread occurrence of daily/circadian, lunar and annual/circannual cycles at almost all levels molecular, cellular, physiological and behavioral and persist in all groups of living organisms. Bunning (1936) pioneered the proposition that ‘photoperiodic effects are based on physiology, lies within the endogenous rhythms’. This means that light affects various life history stages of most animals including seasonal breeders. The annual changes in environmental daylength are reasonably predicted, but availability of food and other environmental factors also modify their annual phenology. Photoperiodic effects through the duration of light, thus of darkness received and mediate the development of long lasting behavioural patterns often related to reproduction and migration. The daily (namely, sleep-wake cycles, locomotor activity etc.) and seasonal rhythms (reproductive cycle etc.) are synchronized by day length throughout in most long-lived organism. Among seasonally breeding animals, birds are a suitable subject of study to investigate the underlying adaptive changes to breeding at most suitable time of year and allow for the birth of young ones at a time optimal for the survival of the young in terms of factors such as ambient temperature, food and water availability, and even changes in the predation behaviors of other species. Avian reproduction is guided by seasonal physiologic controls such as favorable climate, low predation risk, social interaction, and food and nest site availability. Internal physiologic processes work in combination with external factors to promote gonadal development. This annual cycle involves integration between
environmental, physiologic and behavioral conditions. Biological clocks regulate the release of hormones and other chemicals that regulate metabolism, reproduction and behavior. While gonads are fully-grown during breeding season, at other times of the year, they undergo changes like involution. With comparable asymmetry in annual reproductive cycle, seasonality is described in both males and females. Male seasonal breeders exhibit changes in reproductive organs and fertility depending on the time of year. The testes are paired, ellipsoid shaped organs covered with the tunica albuginea. Suspended in mesorchiurn, testes change in size and color in response to hormonal fluctuations that influence sexual activity. The inactive testicles are small while active testicles are significantly with increased number of Leydig cells and mark the nuptial phase of the annual reproductive cycle.

Seasonal breeding is widely studied in birds inhabiting temperate and tropical environments. In mid and high latitudes seasonal changes in daylength are more extensive than low latitudes. The inhabitants in high-latitude environments are thus provided with selective advantage that permits them to survive the severe seasonal changes (e.g., migration etc.). But the daylength changes are less extensive with the decrease in latitude. Wingfield et al., (1993) have suggested that the degree to which populations integrate environmental signals to time gonadotropin secretion leading to gonadal development depends upon the predictability of their breeding seasons. Anyhow, seasonal reproductive events i.e. onset of gonadal growth or recrudescence in a seasonal breeder is triggered only if the animal receives a specific photoperiod length. This is referred to as the critical day length or critical photoperiod some organisms, an action will be triggered when the photoperiod falls below the critical photoperiod.
(short day breeders), whilst in others an action will not occur until the photoperiod had passed the length of critical photoperiod (long day breeders).

Photoperiodic time measurement enables organisms to assess and use day length as an anticipatory cue to time seasonal events in their life histories. Photoperiodism is especially important in initiating physiological and developmental processes that are typically irrevocable and that culminate at a future time, thus acting as a proximate cue for breeding. The proximate cause is what is immediately observed as causing the behavior. The role of day lengths cannot be disregarded when evaluating the mechanism underlying life historical events, range expansion, invasions of novel species, and response to climate change. Endogenous physiological oscillations brings various seasonal activities associated with birds life in a way to cope optimally with the environmental demands imposed on the species such that there is close association between physiological and behavioural functions and the environmental light-dark cycle. While a proximate factor acts to increase fitness, the evolutionary cause is called the ultimate cause. Food is another ultimate factor that affect the anticipation and culmination of breeding events. There is plasticity in food use among geographically dispersed conspecifics (Kramer 2001). It is also suggested that reproductive requirements may also influence consumption patterns (Lahti and Rytkönen 1996). During breeding, male birds often court females with offers of food, or directly feed nestlings. Other factors, such as temperature also act as proximate cue and together with endogenous rhythms, regulate reproductive activity on the physiological level (Gwinner, 1986; Ball, 1993; Cockrem, 1995).

Variability in environment is an important limitation in evolution of organisms (Begon et al., 1996). The Indian subcontinent shows a great diversity
in its topographical and climatic nature. India is home to an extraordinary variety of climatic regions. It extends between 8° 4' and 37° 6' North latitudes and 68° 7' and 97° 25' East longitudes. There are some studies on photoperiodic responses of long day birds inhabiting Indian subcontinent. These birds begin to prepare for breeding with the increasing day lengths in spring (brahminy myna, Sturnus vulgaris- Kumar and Kumar 1993; blackheaded bunting, Emberiza melanocephala-Misra et al., 2004; house sparrow, Passer domesticus- Trivedi et al., 2006) and attain gonadal maturation during the long days in summer, thus categorized as classical long day birds. Subtropical seasonal breeders also include post-monsoon breeders, in which breeding initiates under long days but occurs after the after monsoon. The relevance of day length to natural reproductive cycle of these birds is not clearly known (but see Budki et al., 2012).

Our pilot study has implicated an endogenous sensitivity in the photogonadal responses of a post-monsoon breeder, blackheaded munia (Lonchura malaccana malaccana) (Gupta, 2013). This thesis details studies undertaken to investigate the extent of involvement of the photoperiod with respect to seasonal reproduction and locomotor activity in this bird.

1.2. Experimental Model

The blackheaded munia (Lonchura malaccana malaccana) (plates 1 and 2) also known as tricolored munia, is an estrildid finch. It is small passerine, gregarious bird that feed on grains and other seeds. It has good survival rate under laboratory conditions, there are about eight species of birds under the group called ‘munia’ these are the prettiest group of birds around. India has at least three of those present in vicinity viz.- red munia, spotted munia and
blackheaded munia. They are very common all over India. Since blackheaded munia is a non-migratory resident bird, the results obtained on this species can be compared with other investigations on some tropical and subtropical birds. Some of the results from the studies on munia can be summarized as follows.

1- The spotted munia is non-photoperiodic species in which short days are gonado-stimulatory, an endogenous circadian oscillator regulates its seasonal cycle (Chandola et al., 1980). Further, studies on spotted munia indicate that two environmental correlates - changes in day length and food intake/availability may serve as proximate factors to synchronize breeding in this bird (Chandola et al., 1983). But the customary long and short days (ranging 8-24h) fail to stimulate the gonadal growth, long day rather retard or inhibit it. In tropical blackheaded munia both, long and short photoperiod were gonado-stimulatory (Thapliyal and Saxena, 1964; Pandha and Thapliyal, 1969). Though they differed in the time for induction of response (Chandola et al., 1974; 1975).

2- Further when blackheaded munia is subjected to ahermeral cycles (viz. 3L: 23D), they are gonado-stimulatory, though when exposed to 3L: 21D for longer duration the gonadal response coincides annual gonadal cycle in nature (Chandola and Thapliyal, 1977). They argued that the endogenous rhythm of reproduction can override the effect of LD regime used. Whilst, some agree that day length induces gonadal development in this species (Pandha and Thapliyal, 1969) and that regression is caused by some other seasonal factor; others agree that response to photoperiod persisted as ancestral response has become desynchronized (Chandola et al., 1975).

1.3. Structure of Thesis
All the experiments included hereafter have been performed in the male blackheaded munia (*Lonchura malacea malacea*) and referred to as munia. The thesis has been divided into five units.

Unit I include General Introduction with a background around which the study is designed. It discusses the ubiquitous biological rhythms as adaptive strategies. As the present study involves photoperiodism in an Indian resident bird, some information regarding photoperiodism, the Indian subcontinent and the advantages of blackheaded munia as an experimental model.

Unit II comprises a review of literature with a brief survey of different types of present day information on biological clocks.

Unit III deals with General Materials and Methods including information about bird. Methods of data collection including recording of locomotor activity rhythms from experiments conducted under natural illumination conditions and under artificial setting of light-dark conditions and observation of parameters such as body weight, testis size, food intake are described followed by brief update on statistics used to test the reliability of experiments performed.

Unit IV comprises three chapters.

Chapter -1: Seasonality in Reproduction

Chapter- 2: The Response mechanism and photoperiodic time measurement.

Chapter- 3: Locomotor activity rhythms as a marker.

Unit V discusses the summary major findings of thesis work and precedes the references.
The time of sunrise and sunset across the year serves as a valuable index of seasons at given latitudes and longitudes. Daylength is a major source of temporal information regulating seasonal reproduction and associated events in a number of avian species. All terrestrial animals exhibit principally two types of biological variation namely daily and seasonal rhythms, though other celestial bodies especially the revolution of moon around the earth and its position with respect to earth affects various biological timing in tidal animals and hence their life and adaptations.

Living organisms have acquired response systems that are sensitive to timed rhythms of environment and therefore are called the biological clocks. These endogenous clocks are synchronized by geophysical zeitgeber or by endogenous, internal, rhythms. Exogenous stimuli are called Zeitgeber, which means ‘time giver’ in German, and may comprise light, temperature and length of day. Endogenous clocks are internal rhythms that are preprogrammed to correspond with the environmental temporal pattern. An early discovery of regulation of clocks is credited to a French astronomer named Jean Jacques d’Ortous de Marian (1729), who noticed plants that displayed daily leaf movements. He noted that the leaves of the plants continued to open during the day and close at night even when transferred to dark. He concluded that the observed rhythm was not passively driven by a cyclic environment but was an innate property of the plant. More discoveries followed from invertebrates and
vertebrates and the formal studies of biological rhythms were formulated.

2.1. Biological Clocks

Biological clocks are ubiquitous just because rhythmically changing environmental conditions pose continuous challenge to every organism. The existence of clocks ranges from bacteria to humans. Cyanobacteria have photosynthesis during the daytime and Nitrogen fixation during the night. In cyanobacteria, the circadian clock controls many genes. Liu et al., 1995 have shown that the activity of essentially all promoters is rhythmically regulated. The clocks are rather complex single cell organisms to multicellular plants and animals. In terms of time, duration of the repetitive rhythm ranges from a few minutes to months. The present review briefly describes the clock: its properties and seasonality: an Indian perspective.

2.1.1. Importance of Clocks

The significance of such an adaptive strategy lies in providing an opportunity to the organism such that it may regulate the different phases of its life history to best suite the changes the external cyclic environment. Therefore, temporal organization of the species helps it to live most profitably in its environment. Therefore, it is important to be prepared for conditions (either advantageous or disadvantageous) rather than just reacting to them. For any organism that is prey of another, it is vitally important to know at which time of the day predators are most likely around. The same reasons apply for animals inhabiting the seashore in order to know when the tide is low or high. Biological clocks reflect rhythms caused by the interplay of sun, moon, and earth make it possible to anticipate forthcoming changes in the environment.

2.1.2. Types
Daily and annual cycles are most observed forms of rhythms among vertebrates. Recurrence of daily rhythmicity in a myriad of functions, from metabolism and physiology to behaviour is known as circadian (circa-about, dies-day) rhythm. Under constant condition e.g. of light or darkness, organisms exhibit their endogenous rhythmicity with period (\(\text{tau} \,[\text{t}]\)- the interval between two consecutive identical phases of the oscillation) close, but not exact, to 24h.

Many animals display physiological changes throughout the year interpreted as seasonal behaviors in long-lived organisms, ranging from insects to mammals. These are called circannual (circa about; annum year) rhythms since under constant conditions organism exhibit their endogenous rhythmicity with period close, but not exact, to one year. Just as circadian rhythms allow organisms to anticipate daily Zeitgeber cycles (German word, zeit-time; geber-giver, e.g. light-dark [LD] cycle), circannual rhythms allow them to predict and respond to seasonal cycles of the environment. Circannual rhythmicity has been demonstrated in plants and many animal species like mammals, birds, reptiles, arthropods, and mollusks comprising functions like hibernation, migratory behaviour, locomotor activity, and hormonal status (Gwinner, 1981).

Many marine species are affected by tidal changes resulting from gravitational and centrifugal forces generated from the interplay of earth, moon, and sun. Terrestrial species that inhabit tidal regions have to deal with high and low water levels. Adaptation to moon phases can be observed for twilight and night active animals although these rhythms are in most cases merely passive reactions to the amount of light changing from new moon to full moon. There are suggestions that the female menstruation cycle synchronizes with moon phases but this is rather synchronization to social or olfactory stimuli than caused by an
endogenous circa-lunar clock (Roenneberg, 1998).

Although circadian rhythms are the most extensively investigated biological rhythms because almost all organisms, cyanobacteria, fungi, plants, and animals, including humans, are affected by the daily change of light and darkness. The present study also revolves around the theme of circadian rhythms.

2.1.3. Synchronizer

Rhythms can either purely be driven, which means that they would not exist without an exogenous pace-setting signal. Other rhythms may however persist cycling, without fresh stimulation, with a period close to that of the initial stimulus. As the period of these (free running) endogenous rhythms is nearly equal to the period of the exogenous signal, they are called circa (=about) rhythms. Halberg in 1959 for daily rhythms first proposed this terminology, however, it is applied for all rhythms with environmental counterpart. Many species use annual cycle of day length (photoperiod) as a calendar to time their seasonal physiological and behavioral functions. Mostly, if not universally, daylength regulates a wide spectrum of physiological and behavioral functions in many species. In keeping with this fact, the role of photoperiod in regulating gonadal development cycle has been clearly established in temperate zone and migratory bird while gonadal stimulatory role of photoperiod is not well known in tropical birds, irrespective of distribution. Most seasonal breeders particularly those living close to equator or in tropics face lesser seasonal variation in day length, than the temperate regions, still exhibit clear seasonality in reproduction and molt. There is great diversity in resident species of birds in India that render a great opportunity and diversity in the study of reproduction and ecology of Indian avifauna.
The most important environmental influences on annual rhythmicity are photoperiod (ratio of day and night length) and temperature. Some mammals become reproductively active with increasing day length (long day breeders, e.g. many rodents) while others are reproductively active during the short day period of the year (short day breeders, e.g. sheep). The state of reproduction is most probably mediated by melatonin secretion, which encodes day length information (Goldman, 2001; Wehr 2001). In birds, molt appears every year even when photoperiod and temperature are kept constant, also testicular width follows a circannual rhythm. Both exhibit a period shorter than one year (Gwinner, 2003). Hibernation of ground squirrels occurs about once a year when animals are kept in conditions like constant darkness (DD), constant light (LL) or in a constant cycle of 12h of light and 12h of darkness (LD 12:12). Again, rhythmicity observed was shorter than 12 months. In general, persisting circannual rhythms can vary greatly, ranging from 7 to 15 months, depending on species. But even within individual species, considerable variation can be observed (Gwinner, 1981).

2.1.4. Components

A biological clock is a conceptualized axiom built on input-pacemaker-output pathway of oscillating systems. Biological clocks are therefore, often compared to technical oscillators (the term clock is used synonymously with biological and mechanical oscillators and zeitgeber with time giver for exogenous stimulus). The pacemaker generates rhythmic oscillations of events (viz. gene expression or hormone secretion etc.) with a precise duration. Rusak and Zucker, 1979 discovered the “circadian pacemaker”- suprachiasmatic nucleus (SCN) in the mammalian hypothalamus in 1972. In fish, reptiles and
birds, discoveries suggested independent clocks in the eye and the pineal gland. Among invertebrates circadian pacemaker appear to be located in the eyes of marine mollusks (e.g. Aplysia and Bulia) and the lateral neurons of Drosophila. All pacemakers however are cellular processes of neural origin. The input pathway perceives stimulus from the surrounding. These comprise a photoreceptor containing light sensitive molecules albeit other non-photonic input pathways. Output is generated as the consequence of underlying gene activity through coordinated control of input, the pacemaker and the output expresses at behavioural (e.g. sleep wake cycles, locomotion) and physiological (e.g. food cycle, secretions of hormones etc.) levels.

2.1.5. Properties

As discussed, in the absence of any synchronizing zeitgeber, the self sustained clock "runs free", i.e., it cycles with its own inherent, endogenous period $\tau$ (tau). If a clock is entrained, the free running period adjusts to the period $T$ of the entraining zeitgeber ($\tau = T$). Jürgen Aschoff showed this for humans through bunker experiments (Aschoff, 1965). Entrainment is a process that systematically functions depending on- a) the period, b) the duration, and c) the strength of the entraining zeitgeber, furthermore, d) period and amplitude and e) the responsiveness of the clock (Roenneberg et al., 2005).

2.1.6. The Two Models of Entrainment

In order to adjust an oscillator to a rhythmic zeitgeber, the strength of the zeitgeber and the phase of the endogenous clock cycle are of major importance. This somehow mixes up the two basic models of entrainment. The continuous model proposes that the intensity of light, as the most important zeitgeber in nature, is mainly responsible for changes in phase angle and free running period,
which leads to an entrained rhythm. The model is mainly based on the
observation that free running period under constant light conditions (LL) changes
with increasing light intensity in many species. This observation is called
Aschoff rule, and Jürgen Aschoff (1965) was an advocate of this model. Another
pioneer of chronobiology, Colin S. Pittendrigh (1996), supported the discrete
model, which claims that individual entraining pulses are the basis for
entrainment. Experiments with rodents showed that time point of application and
not intensity of light is important to obtain stable phase angles (Decoursey,
1972). For this model, only free running period and phase response curve (PRC)
are important. In LD, mice are active during darkness and stop moving as soon
as light is switched on. To determine the "real" end of activity triggered by the
clock, the mice were, after being entrained for several days, released into
constant darkness (DD) followed by a re-exposure to LD cycle to test if it was an
actual free-run or masking. This tested whether observed free-run was acute
response to light than an output of the entrained endogenous clock.

2.1.7. Circadian Photoreception

Rods and cones in the retina of eye were earlier considered as the receptors for
circadian light input. Surprisingly though, loss of rods and cones could not
abolish entrainment ability of mice in a study by Lucas et al. (1999), in which
they investigated if retinal structures were necessary for the adaptation of the
circadian system to light. More so complete loss of the eyes, abolished
entrainment to light. It was then found that a small subset (~1%) of retinal
ganglion cells (RGCs) is directly (intrinsically) photosensitive and probably acts
as primary photoreceptors for the circadian system. In these ganglion cells,
melanopsin is expressed (Berson et al., 2002), an opsins protein (Bellingham and
Foster, 2002) first discovered in melanophores of *Xenopus laevis* and subsequently identified in the human inner retina (Provencio et al., 1998; 2000). Mice lacking melanopsin and rods and cones show no significant pupil reflex and fail to entrain to light/dark cycles (Panda et al., 2003). Photosensitive RGCs constantly express melanopsin to enable photosensitivity (Melyan et al., 2005). The photo-response of RGCs in macaques, primates with a visual system similar to humans, is like that of rodents (Dacey et al., 2005) and a major role of the RGCs could be the detection of twilight (Foster 2005), the transition between day and night with different wavelengths than daylight (Roenneberg and Foster, 1997) and thus a potential entraining signal. In birds, the early expression onset of nonvisual photo-transduction molecules confers premature photosensitivity to RGCs (Verra et al., 2011). They have further suggested the identification of a novel type of photosensitive cell through appearance of Opn4x expression in horizontal cells.

2.2. **Seasonality: An Indian Perspective**

Indian subcontinent stretches between tropical and subtropical belts. Physically, country is divided into four relatively well-defined regions, viz. a) Himalayan Mountain; b) Gangetic river plains; c) southern (Deccan) plateau and d) Islands of Lakshadweep, Andaman and Nicobar. The Himalayan ranges, that comprises highest peak in north and tropical rain forest in south, which impart a great degree of isolation and enormous complexity to geophysical characteristics. The northern plains of India stretch from Assam in the east to Punjab in the west. The plains are remarkably homogenous topographically. Similarly, while the west of Indian subcontinent comprises mostly deserts, the east has a great range of vast mangrove forests. Mahindra (1942) has subdivided Indian peninsula into
following subdivisions. Western Himalayas, eastern Himalayas, West Indian deserts, Gangetic plains, Assam, central India, Malabar, Deccan, and Andaman. Its climate is mainly modified by oceans and mountains and is characterized by variety of climate type. It is the outcome of extraordinary pressure conditions that develop in central Asia during summer and winter seasons. The word monsoon is derived from Arabic word ‘mausam’ which means seasons. It implies seasonal change of prevailing winds. During winter, the prevailing winds are offshore from sea towards land. During summer, these winds blow on-shore from sea towards land. This change from land winds to sea winds and vice versa is the cause for monsoon climate. The climate of India defies easy generalization, comprising a wide range of weather conditions across a large geographic scale and varied topography. Analyzed according to the Köppen system, India hosts six major climatic subtypes, ranging from desert in the west, to alpine tundra and glaciers in the north, to humid tropical regions supporting rain forests in the southwest and the island territories. Many regions have starkly different microclimates. The nation has four seasons: winter (January and February), summer (March to May), and subsequently followed by spring and monsoon. The Himalayas act as a barrier to the frigid katabatic winds flowing down from Central Asia. Thus, north India is kept warm monsoon (rainy) season (June to September), and a post-monsoon period (October to December). The tropic of cancer passes through the subcontinent, thus, dividing India into subtropical and tropical region. The whole country is considered to be tropical. India is home to an extraordinary variety of climatic regions. India’s unique geography and geology strongly influence its climate; this is particularly true of the Himalayas.
or only mildly cold during winter; in summer, the same phenomenon makes India relatively hot.

In winter the average temperature, in the subtropics is $16^\circ$ C and that in the tropical India $25^\circ$C. During winter, there is more sunshine in the southern hemisphere due to tilting of earth's axis, while the angles of sunrays become less in northern hemisphere. In the month of February, when sunrays start falling on the northern hemisphere more directly, Indian subcontinent grows warmer and spring season sets in. Rainfall throughout the subcontinent is received during monsoon; such that the climate of India is dominated by Asiatic monsoon i.e. rains from southwest between June and October. Even the arrival of monsoon shows parity in different geographical locations.

Monsoon (Southwest) arrives in peninsular India in June and leaves latest by October. In the Northern parts, rain arrives in and leaves by September. The whole subcontinent experiences drier winds from North between December and February. From March to May climate is dry and hot. The temperature increases from south to north and northwest in summer. It begins from April and extends up to June. It is marked by an appreciable rise of temperature and decreases of barometric pressure.

Because of great diversity in climatic nature, there is marked variation in distribution of natural vegetation of the land. The flora and fauna is also correspondingly of different type. The natural floral cover in Himalayan region varies with altitude and comprises evergreen forest with alpine meadow while rain soaked foot hills contain deciduous trees and grass with patches of tea plantation and florescent green rice fields. Between the north south extremes, the country has semi-evergreen rainforests, deciduous forests, and subtropical pine
forests in the lower mountain zone and temperate mountain forest. Due to enormous complexity and habitat diversity it renders possible study of reproduction in a wide variety of avifauna of similar habitat and similar/same species in diverse habitat. This provides an excellent opportunity for simultaneous analysis of various ecological factors operating.

2.2.1. Seasonality in Animals

Daylength regulates a wide spectrum of physiological and behavioural functions in most species inhabiting Indian subcontinent. However, the timing of the seasonal events in birds in India also depends on the geomorphic characters, climate and proximate factors like food availability etc.

It has been document in rotifers, annelids, molluscs, arthropods, echinoderms, bony fish, frog, turtles, lizards, birds and mammals. In general, photoperiod provides the go/no-go cue for the direct timing of seasonal events, or for the initiation of physiological, endocrine and developmental cascades that, once started, are irreversible or, at least under natural conditions, usually not reversed before the completion of seasonal events. Photoperiodism provides the most important zeitgeber (setting agent) of circannual rhythms (Gwinner and Helm, 2003).

2.2.2. Role of Photoperiod in Regulating Seasonality in Birds

Any predictable change or pattern in time series that recurs or repeats over or one-year period can said to be seasonal. Seasonality refers to the part of year, when a particular activity takes place. Most species of bird exhibit well-defined seasonality in their physiological and behavioural functions like reproduction, molt, bill colour hormones levels, song production etc. Most of these functions generally center on reproduction that occurs at most appropriate
time of the year, when the food resources in the wild are optimally present and the chance of survival of young ones and parents are at their maximum. Therefore, the timing of actual reproduction is critical for the species.

In birds, the annual change in day length is most important environmental cue used for synchronizing breeding, molt and migration, with recurrent seasonal fluctuation in environmental conditions (Coppack and Pulido, 2004). For birds with their flight adaptations, it is costly to maintain reproductive systems at full capacity. Therefore, these systems atrophy seasonally in seasonal breeders. Among early studies, Rowan (1925) demonstrated the role of vernal increase in day length, in reproduction in birds to show that daylength regulates seasonal change in the body mass, gonadal growth and molt (Farner and Follett, 1979; Wingfield and Farner, 1980; Brandstatter et al., 2000; Dawson et al., 2001; Misra et al, 2004). Because 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. The role of photoperiod in regulating annual gonadal cycle has been clearly established in temperate zone and migratory birds. Temperate birds must coordinate several key events into their phenology, principally reproduction, molting and migration (Dawson et al., 2001; Gwinner, 1996). These processes are mutually exclusive energetic activities and are sequentially orchestrated in the seasonal life history of birds, both in nature and as circannual rhythms under constant conditions. But, gonadal stimulatory role of photoperiod is not well known in tropical birds. Most species, particularly those living close to equator, or in tropics, having either no or very little seasonal variation in day length, show clear seasonality in reproduction and molt. 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. This possibility has been examined in some tropical and subtropical species and it has been found that in spite of small photo-fluctuation, light plays a much more significant role than has hitherto been assumed. These animals have been shown to exhibit high sensitivity to small changes in daylight duration and intensity. The response to any given daylength depends on the precise phase of the annual or circannual cycle (Helm et al., 2009).

2.2.3. Studies on Indian Birds

Professor A. B. Mishra was first to initiate work on avian reproduction. Work based mostly on size and histology of gonad (Misra, 1948). Thereafter, Thapliyal and his colleagues worked on about 30 wild species of birds and demonstrated predictability in annual sexual and body weight cycle of these species (see reviews Thapliyal, 1978; 1981). In tropics and subtropics, many avian species appear to use day lengths as information for timing their breeding cycles (Thapliyal and Saxena, 1964; Thapliyal, 1980; Singh and Chandola, 1981; Chaturvedi and Thapliyal, 1980). Henceforth, it is now established that the seasonality in reproduction is also well marked in birds in tropics despite the fact that variation in photoperiod and temperature across the year is less pronounced as compared to that in temperate zone. However, in monsoon season there is a selective advantage of breeding as it is favored by maximum availability of food in nature. Experimental investigation of factor (external and internal) that may regulate reproduction in birds started with the publication of reports that in lal munia (Estrilda amandava) and in Indian weaver bird (Ploceus philippinus) plumage pigmentation cycle run almost parallel with the gonad development.
cycles and are regulated by the circulating levels of the pituitary luteinizing hormone in number of other finches these are controlled by genetic factors (Tewary and Thapliyal, 1962).

**2.2.4. Photoperiodic Time Measurement**

Endogenous circa rhythms believed to be involved in photoperiodism are synchronized to changes in illumination of environment with either time of day or season of the year i.e. (circadian and circannual rhythms respectively) whilst the period and phase of daily light dark cycle is key to synchronization of the circadian timing system, the annual photoperiodic cycle ensures synchronization of the circannual system to seasons as year.

As such, in all photoperiodic vertebrates investigated, one part of 24h day is especially sensitive to presence of light and whether or not this photosensitive phase (photo-inducible phase) is illuminated determines whether a day is registered as ‘long’ or ‘short’. The extent to which it is a component of circadian system varies considerably and there is a spectrum of responses. Thus, the extent to which the photoperiodic pacemaker self sustains (free runs in darkness) varies from species to species. While it is strongly self sustaining in the European starling (Juss et al., 1995); house finch (Hamner and Enright, 1967); hamster (Elliot et al., 1972; black headed bunting (Singh et al., 2002); it is weakly self-sustaining in Japanese quail (Saiovici et al., 1987, Juss et al., 1995). Much less has been done to describe the nature of photoperiodic oscillator in an Indian resident species of birds, and lesser in post monsoon breeding birds.

Animals have evolved many season-specific behavioural and physiological adaptations that allow them to both cope with and exploit the cyclic annual environment. Two classes of endogenous annual timekeeping
mechanisms enable animals to track, anticipate and prepare for the seasons: a
timer that measures an interval of several months and a clock that oscillates with
a period of approximately a year. The basic properties and biological substrates
of these timekeeping mechanisms, as well as their reliance on, and encoding of
environmental cues to accurately time seasonal events.

There are some propositions regarding the mechanisms animals use to
achieve seasonally appropriate adjustments. Although reference is mostly made
to studies of reproduction and to particular species, the conclusions extend to a
broad spectrum of animals and to non reproductive traits.

Using the hypothesis of Bunning, we study a photoinducible phase during
diurnal rhythm. Attempt to determine a photoinducible phase for gonadal
stimulation during the diurnal rhythm i.e. How can the physiological system
measure the daily variation of illumination?

Among the most explicit theories stated so far, the most explicit theories
are those of Bunning (1960) and Pittendrigh and Minis (1971). These authors
have suggested that there is an endogenous daily rhythm in photosensitivity; this
rhythm is imagined as having two halves per cycle: during one time the organism
is light « insensitive », and during the other it becomes « sensitive »- plate 3

A physiological process is stimulated only if light coincides with the
sensitive phase of the daily cycle. In winter, the photoperiods are too short to
reach the sensitive phase but, as they lengthen in spring, light begins to
progressively reach the phase, and so automatically to stimulate the physiological
process. The existence of such a photoinducible phase, determined in relation to
dawn, has been shown in birds. The working hypothesis is explained on the basis
of internal and external coincidence models (plate 4). But before mentioning
those models, a mention of hourglass model by Garner and Allard, in 1920, suggested for photoperiodism in plants. The hourglass model assumes the gradual accumulation of a chemical product in the organism. This model argues against the involvement of the circadian clock in the photoperiodic response because the hourglass lacks endogenous rhythmicity and must be reset or "turned over" by the light cycle each day. The involvement of the circadian system was, however, detected in almost all species studied to date, so the hourglass mechanism is unlikely to have evolved in real organisms.

The external coincidence model was proposed by one of the pioneers of chronobiology, Erwin Bunning, in 1936. This model proposed the existence of a circadian rhythm of photoperiodic photosensitivity (CRPP) in which most of the night-phase is sensitive to light, while the day-phase is photo-insensitive. As the day gets longer in spring, light starts illuminating the photosensitive phase and triggers the physiological or behavioral response. In this model light has a dual effect: it entrains the rhythm of photosensitivity and also acts as the stimulus (that is stimulates a photoperiodic response) if the light falls on a photosensitive phase of the rhythm of photoperiodic sensitivity. This model is termed an "external coincidence" model because it requires the coincidence of an external stimulus (light) with an internal rhythm of sensitivity to light.

This dual role of light is not necessary if one considers the "internal coincidence" model, proposed by Colin Pittendrigh and Dorothea Minis in 1964. In this model, the light's only role is to entrain the circadian system. At the time this model was proposed, it was becoming apparent that multicellular organisms house more than one circadian pacemaker. Each of the oscillators will
behave differently under the influence of the light-dark cycles, and assume different phase-relationships with the entraining cycle.

Pittendrigh and Minis proposed that changing photoperiods might alter the internal phase-relationships between two or more rhythms, bringing them into permissive (red) or inhibitory modes. For example, secretion of a hormone has to coincide with the availability of its receptors at the target tissue, or with the absence of the enzyme that metabolizes it. In this model, therefore, light must control only the internal phase-relationships between multiple circadian rhythms.

In simple or natural 24 h photoperiods, a light sensitive or photoinducible phase (ϕ) falls in the dark when the photo-phase is short (or the scoto-phase long) but is illuminated when the photo-phase becomes longer (or the scoto-phase becomes shorter) than the critical value. The seasonally appropriate switch in metabolism, which leads to the appearance of physiological stimulation, is thus attributed to changing phase relationships established between oscillators and the daily light cycle during entrainment. The control system of the internal oscillator, which plays an essential role in the proposed photoperiodism explanation, is a derivation of the feedback model.

In view of the above explanations, Munia does not fit into classical long day or short day “designations” and is therefore different in its photoperiodic responses as compared to other classical long day species, it may be possible that it has or does not have a photoinducible phase for gonadal stimulation, which is explained in the few other birds. This way it would just be appropriate to seek to understand the mechanism regulating circannual rhythm in this bird to enhance our current understanding of wide spectrum of responses of Indian resident birds.
3.1. Model of Study - Blackheaded Munia

All experiments included in the study were performed on adult male birds of *Lonchura malacca malacca*, a bird that belongs to family Estrildinae (a family of munias) of order Passeriformes of the class (Aves). Some of the species of Blackheaded munia are - *Lonchura malacca rubronigr*; *L. m. Sinesis*; *L. m. batakana*; *L. m. formosana*; *L. m. deignani*; *L. m. brunneiceps*; *L. m. jagori*; *L. m. selimbaue*; *L. m. obscura*; *L. m. maja*. These species have worldwide distribution. They occur commonly in India and the following species are seen in paddy fields as the vegetation appears in its boom - *L. m. oryzvora* (Java sparrow); *L. punctulata* (Scaly breasted munia); *L. striata* (White rumped munia); *L. kelwarti* (Black throated munia). Blackheaded munia species is commonly known as Nukroul.

3.1.1. Bird’s Details

*Lonchura malacca malacca* (Plate 1) is regionally addressed by different common name(s), such as Tri-coloured nun, tri-coloured munia, three-coloured mannikin. These birds are distributed in India and Sri Lanka and it is believed that their origin is in Sri Lanka or South of India. In wild, they live among tall grasses and reeds, wet tropical environment and have adapted to farmlands such as rice paddies and sugar cane farms. In wild, they are secure and sturdy birds. Their age from the time of hatch to sexual maturity is about 6 months, while the adult plumage is attained at about 6 - 12 months. The birds has a life span up to 8
years but on an average, best breeding years are considered to be 2nd to 5th year. But in most cases, a bird can breed from 12 months to 5 years.

There is no sexual dimorphism in blackheaded munia.

Local bird catchers made adult birds available. Average length of a male bird is approximately 115 mm (or about 4.5 inches) and weighs approximately 20 gms (or about 2/3 oz). Black headed Nun, Silver headed Nun or the Tri coloured Nun have similar husbandry requirements.

3.1.2. Distribution

The Estrildid finches are small passerine birds of the old world tropics (Plate 2) and Australia. They are gregarious and often colonial seed - eaters with short thick but pointed bills. They are all similar in structure and habits, but a wide variation in plumage colors and patterns. It is a locally common resident bird that shows some monsoon dependent local movements. These are largely distributed in India, Bangladesh, Brunei, Cambodia, China, Indonesia, Laos, Malaysia, Burma, Nepal, Philippines, Singapore, Taiwan, Thailand and Vietnam. In India it is largely distributed Raipur, Pachmarhi and Mumbai up to Kanyakumari and Srilanka and in plains and lower hills up to 2100 meter in India.

3.1.3. Habit and Habitat

Being a resident in the Indian sub-continent and neighbouring lands, this bird affects marshlands reed beds, grass fields. This is a lowland open country bird. The bird also pick up food on barren grounds and often a ground feeder. It occurs in open forests, scrub jungles, swampy grassland and neighbourhood of flooded paddy cultivation.
Blackheaded munia is 11-12 cm in length. The adult has stubby pale gray bill, head is black while the rest of upper parts are Rufus chestnut coloured. Rump is dark. Throat, breast and centre of belly are black, under rest of under parts are white. But juvenile birds have uniform pale brown upperparts lack the dark head and have white to pale buff under parts. These birds generally prefer wetlands, ill drained area and become very active especially during paddy cultivation. However, the bird feeds on egg diet (boiled and mashed eggs) mixed with bread crumbs under laboratory conditions.

3.1.4. Social Behaviour of Blackheaded Munia

This lowland bird is known to move in larger flock thus attracting poachers to harvest them in big numbers for resale in the pet shop. Though easy to spot in the padi fields and grassland, they are not encountered so often. They have all gathered in specific spots and not scattered. the large flock and continued presence makes the scene tempting for poacher who will find it profitable to harvest a good number with a single efforts.

The birds moves in small flock of 10-30 birds, feeding on the ground particularly those areas with some water sources like drain and springs. Blackheaded munia is docile, hardy, long lived bird. These are social birds that like to lives in flock and fond of company of its allied species, marshland hunting munia and frequent associate of Ploceus manyar (Weaver) in breeding seasons in south India. In nonbreeding period, they keep in flocks of up to 100 and more individuals some times in company with spotted munia. These are sensitive to cold and require a warm, usually tropical habitat. They can be kept as a colony or as a single pair in a mixed finch collection. Sometimes, munia cause serious damage to standing crops. The food chiefly comprises seeds and grains (namely
bajra, rice, wheat, jowar etc). Feed on grounds, flying in undulating rabbles up into tree tops when distributed, soon descending again in two's and three's to resume feeding feeds on grass seeds and rice. Flight call is a triple chirp.

3.1.5. Procurement and Maintenance

Adult black-headed munia were obtained through local suppliers or Lucknow- Nakkash during May to September, every year. These were acclimated to laboratory conditions, in an outdoor aviary for 3 weeks. Later, they were caged in groups of up to 6 individuals per cage. The indoor facility inside the room received unrestricted natural illumination through large windows with a glass pane. Food (seeds of Kakuni, *Setaria italica*) and water were provided *ad libitum* and replenished only during the period of light on. All birds used throughout the experimentation were kept in similar husbandry conditions. All the experiments were in accordance with the guidelines of institutional (Chaudhary Charan Singh University, Meerut) ethical committee on experimentation on animals. Food and water was replenished daily and water as replaced every day for stock birds during study.

During the photoperiodic experiments, the birds were held in groups of 4- 5 individuals per cage (size 45 X 25 X 25 cm³) in lightproof boxes (size 1 X 1 X1m³) provided with white compact fluorescent lamps at ~500 lux. Automatic time switches were used to control periods of light and darkness. Light intensity was measured regularly at perch levels by a digital lux meter. The photoperiodic chambers were well aerated and the temperature inside these chambers did not vary more than 1-2°C from the room temperature. However, we recorded the temperature of outdoor aviary at least daily.

3.2. Methods of Data Collection
Birds were housed singly or in groups in wire net cages as per requirement of the experiment. Body weight and food intake were taken as physiological markers while the transition of various life history stages was studied through observation of changes in testicular volume and the changes in plumage condition as a result of photoperiodic treatment or food alteration as per the requirement of the experiment.

3.2.1. Body Weight

To record the body weight, each bird was individually wrapped in a cotton bag and weighed on a portable top pan balance to nearest of 0.1g. Later, the weight of the bag was subtracted from total weight to determine the weight of each bird. During an experiment birds were weighed on a weekly basis.

3.2.2. Testis Size

The changes in testis were assessed as testicular volume. For this, the dimension of left testis of each bird was recorded by unilateral laparotomy performed using local anesthesia. A small incision was made between the two last ribs on the left flank and the left testis was located within the abdominal cavity with the help of a spatula. The length and width of testis was estimated using reference sets of testis, testis volume was calculated using formula \(4/3\pi ab^2\), where \(a\) and \(b\) denote half of the long (length) and short (width) axes, respectively (Boswell, 1991). The error in this method never exceeded 20%. Incision was stitched and an antibacterial medicine (Soframycin ointment, Aventis Pharma Limited, Pune) was applied. In general, infection did not occur in particular area and rapid healing occurred.

3.2.3. Food Intake
Birds were housed singly in wire net cages (size 45 X25 X25cm). Water was provided ad libitum. A fixed amount of food (25 gm) was given to each bird singly housed in the wire net cage. These cages were lined with transparent white polythene sheets on sides up to 5 cms above the perch level and placed in steel trays facilitating spillage collection. They were 20 cm apart from each other and kept on a cemented shelf situated at a height of 1 m in the NDL room. Birds though visually isolated, could still hear one another. The residual food was collected every fourth day and the food grains, which were spilt by the bird in the steel tray, were also carefully collected and added to the residual food. Thus the entire the balance food was weighed and again the fixed amount of food was dispensed in food cups. The difference between the initial and final weights of food cup gave the amount of food consumed by a bird over 72-hour period. The amount of food consumed in each day was calculated as follow:

Total food consumed = Initial weight (weight of cup+ 25 gm. food) – final weight (weight of cup+ food left after removal of feces).

Average food intake/ bird/ gram/ day = Total food consumed

3

Time of food supply and food availability, were varied according to the experimental schedule in the experiments involving different photoperiodic regimes.

3.2.4. Activity Rhythm Recording

To perch hopping activity of individual birds, birds were housed separately in activity cages (see also Malik et al., 2004). Each activity cage had two perches to facilitate bird flight and an infrared sensor system (Napoleon
Maximum 8, PET Immunity sensor, India) mounted in front on a tin panel to
detect the movement of bird within cage. Thus, flight activity of each bird within
the cage was monitored, recorded in 5-min bins and then transmitted to a
computer installed with a data-logging system, Chronobiology Kit (Stanford
Software System, USA). Details of the data logging are mentioned in section
6.4.1.

3.2.5. Statistics

Data are represented as mean ± Standard error (SE). The data was
analyzed using one- or two-way analysis of variance (ANOVA) with or without
repeated measures, followed by Neuman-keul’s post-hoc test if ANOVA
indicated a significant difference. One way repeated measure ANOVA was used
to compare data generated from the same group as a function of time, and One-
way ANOVA was used to compare data of different groups at one observation.
Two-way ANOVA was used to analyze data for any two factors considered
together. The birds, if any, died during the course of experiment, were excluded
from the statistical analysis. Prism Graph pad V was used to analyze the data and
significance was taken at P<0.05.