SUMMARY

According Koskimies, the birds are bioindicators of environment. Most of avian fauna are the best examples of seasonality in vertebrates. The seasonality, represented by the initiation-termination and re-initiation of physiological processes, it is a compulsory adaptation for survival in various species. Almost all bird species that have been studied, exhibit seasonality in several functions, such as food intake, body mass, sleep-wake cycle, reproduction, gonadal recrudescence and regression, molt etc (Wingfield and Farner, 1993; Jain and Kumar, 1995; Kumar, 1997). In regulation of seasonality there two mechanisms appear involved. One is the photoperiodism, and other is the circannual rhythm. According Hillman (1979) photoperiodism is the control of some aspect of life cycle by the timing of light and darkness and circannual rhythms are self-sustained endogenous rhythmicity of approximately 1-year times these component events. These changes are species specific and timed to occur such that birds reproduce at the time of the year when food is optimally available and conditions are suitable for the survival.

Most species of birds breed during late spring and summer of the year, the breeding season among mid- and low-latitude species can be scattered. In tropics, breeding seasons can be found spread out over the entire year although individual species or different populations of the same species are essentially seasonal (Chandola-Saklani *et al.,* 1983). Many tropical birds do breed during spring and summer exhibiting cyclicity in reproduction, similar to temperate species (Gwinner and Dittami, 1984). Stress is a big factor in determining the overall health of our birds. Stress depletes nutrients from the body rapidly and the immune system

becomes depressed. This stress factor effects the whole body respiratory infections, allergies, eating disorders, skin and feather problems are a few of the outward symptoms of stress. It also leads to hormonal imbalances of adrenal, pituitary, thyroid, thymus etc. that further interferes with immune function.

This thesis includes investigations on light intensity related photoperiodic and seasonal responses of adult blackheaded munia (*Lonchura malacca malacca*). The emphasis is layed on studying photoperiodic and seasonal responses of captive blackheaded munia, both under natural daylight (NDL) and programmed artificial photoperiods for short and long term.

This section deals the investigation of food availability effects to the perception and synchronization of circadian activity pattern and food utilization. Photoperiod influence the seasonal behavior. This study was completed in two parts.

Study 1A: Effect of food deprivation on circadian activity rhythm

Food availability affects on the circadian properties (locomotor activity), food intake and gonadal growth in munia birds (*Lonchura malacca malacca*) exposed to 12L:12D light–dark cycle. Two groups of male and female munia (n = 5 pairs each) were exposed to 12L: 12D for day 1-7. On 8^{th} day, group one received 4 h food and group two received food *ad libitum* (24 h). Light intensity was given 260 lux in day time and at 0.3 lux in dark phase. Locomotor activity pattern and gonadal growth were recorded over the experiment period of one year). Results demonstrate that the blackheaded munia has the daily locomotor activity and showed significantly high activity on light phase and completely diminished activity during dark phase so, the black-headed munia showed a complete diurnal rhythm. During food restriction (ZT: 0–4) phase at day 8, activity of these birds declined gradually from dawn to dusk and

statistically significant difference occurred on food ad libitum group (P= 0.0231; unpaired student's t-test). Testes failed to under growth-regression cycle in the birds held under food restricted but a slight induction in testicular growth was observed between days 120–180. Under similar photoperiod, testes were significant larger on the birds of food *ad libitum* group than food restricted at day 90–180 (P< 0.05; Bonferroni *post test*) and there was no significant maturation on ovarian follicles were noticed the food restricted group during entire period of experiment, but significant follicular growth was observed on the birds of food *adlibitum* group on day 150 ($F_{6,24}$ = 25.98, P < 0.0001; 1-Way RM ANOVA) (figure 4d).

Study 1B: <u>To analyse seasonal rhythmicity in gonadal cycle</u>

This study includes results from the experiments that investigated whether photoperiod affects the physiological processes and photoperiodic inductions of seasonal responses in the blackheaded munia during pre-breeding and post-breeding phase of life history stages. Here, we describe changes in body mass, gonadal growth/ development, and molt in body and primary wing feathers of blackheaded munia during the period of pre-breeding phase [winter solstices (December), or vernal equinox (March), or summer solstices (June) breeding phase and in post–breeding phase September (Autumnal equinoxes) and of the year, compared with these observations on birds held captives under natural day length (NDL).

Results demonstrate that mean body mass of male birds gradually increased and decreased throughout the experiments of each groups. There was no significant change in the body mass in June, September, December and March groups [September (SD group) $F_{24,96}$=4.676, P<0.0001 (LD group) $F_{24, 96}$=1.321, P=0.1718; December (SD group) $F_{24, 96}$=0.6141, P=0.9138; (LD group) $F_{24, 96}$=75.73, P=0.7789;

March (SD group) $F_{24,96}$=7.779, P<0.0001 (LD group) $F_{24,96}$=4.680, P<0.0001; June (SD group) $F_{24, 96}$=2.737, P<0.0003 (LD group) $F_{24,96}$=1.429, P=0.1144; 1-way RM ANOVA) (figure 7a, c, e and g). The mean testicular volume in each group attained a peak value and then gradually decreased. There was significant change in the testis volume in June, September, December and March groups [September (SD group), $F_{24, 48}$=10.49, P<0.0001 (LD group) $F_{24,48}$=13.49, P<0.0001; December (SD group) $F_{24,48}$=0.4710, P=0.9186; (LD group) $F_{24,48}$=27.86, P<0.0001; March (SD group) $F_{24,48}$=1.239, P=0.2857 (LD group) $F_{24,48}$=3.947, P<0.0003; June (SD group) $F_{24,48}$=22.76, P<0.0001 (LD group) $F_{24,48}$=4.083, P<0.0002; 1-way RM ANOVA) (figure7 b, d, f and h).

The mean body molt and molt primaries of male birds increased throughout the experiments of each groups (figure 13). There was differential response in body molt of September, December, March and June groups [September (SD group) $F_{6,12}$=1.982, P=0.1476 (LD group) $F_{6,12}$=23.81, P<0.0001; December (SD group) $F_{6,12}$=1.121, P=0.4062 (LD group) $F_{6,12}$=1.625, P=0.2232; March (SD group) $F_{6,12}$=1.055, P=0.4390 (LD group) $F_{6,12}$=2.245, P=0.1099; June (SD group) $F_{6,12}$=3.861, P<0.0222 (LD group) $F_{6,12}$=19.64, P<0.0001; 1-way RM ANOVA) (figure 8a, c, e and g) and molt primaries in September, December, March and June groups [September (SD group) $F_{3,6}$=1.671, P=0.2711; September LD group, $F_{3,6}$=2.399, P=0.1665; December SD group, $F_{3,6}$=6.512, P<0.0257; December LD group, $F_{3,6}$=6.615, P<0.0249; March SD group, $F_{3,6}$=8.614, P<0.0136; March LD group, $F_{3,6}$=3.520, P=0.0887; June SD group, $F_{3,6}$=24.58, P<0.0009; June LD group, $F_{3,6}$=24.58, P<0.0009 1-way RM ANOVA) (figure 8b, d, f and h). In NDL group of September and December month, testis was reduced form, but in March, birds goes

to pre-breeding phase so testes showed the preparatory phase. Seminiferous tubules were narrow and lined by a single or double-layered spermatogonial cells. The intertubular spaces are wide containing interstitial cells. In June month bird was in breeding phase, the seminiferous tubules are highly stretched due to maximum with increase in population of dividing germinal cells, the inter tubular space reduced and seminiferous tubule gradually increased and spermatocytes, spermatogonial cells are present, spermatids and sperm maturation become started. Spermatogenesis is absent in September month and testis regression was started. In LDL groups of September month, testis remains in reduced form due to their photorefractory stage. Seminiferous tubule becomes enlarge and intertubular space reduced in LDL group of December, March and June months. In December group of LDL, bunches of spermatozoa are attached to cells of sertoli in seminiferous tubules. Spermatogenetic activity seems to be maximum in wide lumen. The interstitial tissue between adjacent tubules has become greatly compressed. In June month LDL group, reproductive activity was in peak. Bunches of spermatozoa are present in wide lumen. Intertubular spaces are much reduced. In September, marks beginning of the end of reproductive activity. Regressive changes become much distinct. Tubules are narrow and tunica propria is indistinct. But the end of experiment, SDL groups of September, December, March and June groups, testes were observed in reduced form (Plate I).

Study 2: <u>To investigate responsiveness to changing photoperiods</u>

Two groups of birds (n=20 each) were exposed under changing photoperiodic experiment in (vernal equinox, summer solstice, autumnal equinox, and winter solstice) in a year. A group of 10 birds (male and female) put in sequentially

changing photoperiods, with one hour induction from short day (8 h light per day) to long day (16 h light per day) photoperiod and another group of 10 birds (5 male and 5 female) transfer sequentially one hour deduction from long (16 h light per day) to short day length (8 h light per day).

Results are shown in figure 10. There was significant change occurred in body mass of March, June and December group sequentially transferred to long (LD; 16L:8D) to short day length (SD; 8L:16D) and short (SD; 8L:16D) to long day length (LD; 16L:8D) during March. Changes in testis volume are present in the figure 3d. There was a significant induction in testis volume in birds subjected to March month under LD to SD and SD to LD condition. But the 5 months delayed response in SD to LD sequentially transferred group. In the June month testis of both groups were showed the peak response during the beginning of the experiment and the regression of testis was started after 180 days. In September month transferred group showed the testis recrudescence after 120 days and attain a peak response at 7 months in SD to LD transfer but the testicular growth of LD to SD transfer group remain unchanged during the experiment period. In December month initiation of testicular growth was started after 30 days and attains peak response at 120 days but 180 days delayed response on testis was noticed in SD to LD transfer group. The molt cycle of both the body feathers and wing primaries followed the similar initiation and termination pattern in all transfer groups of LD to SD and SD to LD condition.

There was no significant change occurred in body mass of March, June, September and December group sequentially transferred to long (LD; 16L:8D) to short day length (SD; 8L:16D) and short (8L:16D) to long day length (LD; 16L:8D).

In follicular growth of female birds was noticed March and June transfer group of LD to SD and SD to LD transfer group. But the follicular growth in munia birds remains unchanged in both groups of September and December transfer group. The molt cycle (body feathers and wing primaries) of female blackheaded munia followed the similar initiation and termination pattern as a male blackheaded munia in all transfer groups of LD to SD and SD to LD condition. Histological data of plate II revealed the data of testis volume. Photomicrograph A-D shows transverse sections of SD to LD December transfer group of blackheaded munia. Photomicrograph A shows the initial size of testis. Seminiferous tubules were narrow and lined by a single or double-layered spermatogonial cells. Tunica propria was thin and distinct. Tunica albuginea was fibrous and thick. The intertubular spaces were wide containing interstitial cells. Photomicrograph B shows to pre-breeding phase and size of testis just started to increase. In Photomicrograph C shows the breeding phase, seminiferous tubules are highly stretched due to maximum width, increase in population of dividing germinal cells during the breeding phase. Spermatogonial cells are present but sperm formation was unidentified. Photomicrograph D shows the narrow tubules, tunica propria is indistinct the bunches of spermatozoa are attached to cells of sertoli in seminiferous tubules. Spermatogenetic activity seems to be maximum with wide lumen. The interstitial tissue between adjacent tubules has become greatly compressed. Intertubular spaces are triangular in shape. In the Photomicrograph of E-H shows testis histology of LD to SD December transfer groups, starting of experiment, birds were in pre breeding phase. Their testis was in reduced form. In photomicrograph E, shows the highly reduced seminiferous tubules with complete regression of testis. Intertubular spaces are wider due to the complete reduction of seminiferous tubules.

Tunica albuginea was thick and appeared clearly, In photomicrograph F, the gonadal size were gradually increase and goes to pre-breeding phase, photomicrograph G, testis are in breeding phase, photomicrograph H testis are in post breeding phase seminiferous tubules becomes reduce, inter tubular space increase (Plate II and III).

Plasma is the fluid portion of blood in which the blood cells are suspended. It is a complex mixture of proteins, carbohydrates, lipids, steroids, and free ions whose composition varies with sex, age, starvation and seasons, etc. (Moore, 1948; Clegg *et al.*, 1951; Vanstone *et al.*, 1955; Dessauer and Fox, 1956; Saito, 1957b), to test different photoperiodic stress factor, blood was collected of March group in both (LD to SD and SD to LD transfer) and a September (LD-SD transfer) group. Birds of March group shows pre-breeding phase. No any stress protein was observed, in SD-LD transfer group. The stress factors (130_{kDa} protein bands) were observed in male and female blackheaded munia by LD-SD photoperiodic induction (figure 14). While in post-breeding phase, in month of September LD-SD changing photoperiod, MW123-130_{kDa} stress proteins bands were observed. This stress factor effects the whole body, eating disorders, skin and feather problems are a few of the outward symptoms of stress. When photoperiods were sequentially increased (1 hour in every 30 days) from short day to long day there no stress proteins were found (figure 15).

Study 3: <u>To determine the critical day length</u>

For determining critical day length by different photoperiodic induction, this experiment was performed on 72 photosensitive birds. Birds were divided into eight groups each groups has male and female 8-9 birds and exposed under different photoperiods 9L:15D (group 1), 10L:14D (group 2), 11L:13D (group 3), 12L:12D (group 4), 13L:11D (group 5), 14L:10D (group 6), 15L:9D (group 7) and 16L: 8D

(group 8), for a period of 90 days at 460 lux light intensity in day time and ~ 0.3 lux at night time.

There was no testicular recrudescence in birds of group 1, 2, 3, 4 and 5 exposed under 9L:15D, 10L:14D, 11L:13D, 12L:12D and 13L:11D respectively with 460 lux light intensity, testes remain unchanged throughout the experiment. There was decrease in body mass of all groups at the beginning of the experiment but after 30 days, the body mass of all groups remain unchanged during the entire period of experiment. The testis size of group 7 and 8 started increasing after 30 days and attained their peak on 90 days. Body feather molt and primaries molt regeneration process initiated after 1 week in all groups during except groups 1, 2, 3 and 4 primaries molt which started delay after 5 weeks. Group 1, 2, 3 and 4 birds showed similar initiation response on body molt and molt primaries and its attained maximum response on 10 weeks but did not follow the complete cycle. The duration of molt cycle completed for 8-16 week.

Observation of transverse section of gonads showed the different size under different photoperiodic groups (group 1, 2, 3, 4 and 5), testis was in primitive phase and follicular size was reduced. But in group of 6, 7 and 8, testis volume and follicular diameter increased. For determine photoperiodic stress factor in blood, the sample collected in first week of february and at the end of experiment, in April from all birds of each group, the blood plasma was separated by cold centrifugation, and runs on SDS-PAGE. Out of eight groups of birds four were exposed under long day photoperiod (13L, 14L, 15L and 16L) and remaining four were exposed to short day photoperiod (9L, 10L, 11L and 12L) in month of February, Stress factor's

130_{kDa} protein bands were observed in all blackheaded munia in long photoperiodic induction. While in short day birds no stress protein were observed (figure 21).

Salient points from the studies performed-

1. The photoperiodic responses of blackheaded munia at this latitude (Meerut, India, 29^0N, 77° 45'E) are similar to those of its populations living at higher latitudes. The physiological properties i.e. gonad development, molt and associated secondary sexual characters are influenced by the day length, and this day lengths (photoperiod) are involved in regulation of seasonal cycles. Thus the population of blackheaded munia uses photoperiodic cues from the environment to regulate their reproductive cycle.
2. The food restriction in the environment can influence the circadian and photoperiodic responses.
3. Munia shows distinct seasonality in the gonadal development and molt. The seasonality in body mass is less dramatic however it appears that the day length is involved in regulation of seasonal cycles. Birds are also capable to respond to different light periods. The long day light photoperiodic induction is closer to critical day length.
4. Stress protein appears influencing the long photoperiodic induction and also depends on its reproductive phase of bird.