To test the circadian clock characteristics, activity behaviour of male blackheaded munia was recorded. Two experiments were performed. In experiment 1A, activity of munia was recorded under long days, LD (14L: 10D); and short days, SD (10L: 14D). Locomotor activity of two groups of munia exposed to equinox (12L: 12D) daylength followed by transfer of one group each to continuous dimlight (DD) and continuous bright light (LL) was recorded in experiment 1B. Experiment 2 aimed to describe seasonal trend in daily pattern of activity/rest cycle under natural illumination conditions (NDL). Hourly activity during daytime was more under SD than under LD. Munia did not exhibit bimodality in daily activity pattern; activity during morning, M (2h) was more than evening, E. A free-running activity rhythm was recorded in munia under DD; the same was arrhythmic under LL. The seasonal pattern in daily activity profiles under NDL corresponds to the seasonal changes in daylength. Daylength regulates daily and seasonal activity patterns in blackheaded munia.

Keywords: Activity pattern, Blackheaded munia, Season

Seasonal changes in day and night cycle influence daily changes in the pattern of short-term behavioural functions such as locomotor activity\(^1\). In birds inhabiting mid and low latitudes, the annual cycle of daylength, with systemic changes in its daily duration and amplitude of light and darkness, act as reliable cue to anticipate and adapt to the changes in the environment. The birds are able to assess the rate of change of daylength to time their seasonal events in a subtle manner\(^2\). Besides this, the self-sustained circannual rhythms of endogenous nature are also suggested to regulate seasonal changes in behavioural and physiological processes\(^3,4\). The two mechanisms interact closely being in phase with each other at different times of the year to time the seasonal events. In the house sparrow *Passer domesticus*, a stable and regular daily change in light levels at dawn and dusk provides a reliable indicator of the phase of the day\(^5\). In sparrow, daily twilight can synchronize the circadian activity rhythm but has no role in photoperiodic regulation of testicular cycle\(^6\).

Relative effect of artificial light on seasonal activity patterns depends to some extent on the physiological state of the animal during a annual life history stage\(^6,7\) and food constraints\(^8\). Under natural and artificial illumination, passerines exhibit bimodality in their daily activity patterns. This bimodality is a product of relationship between two component oscillators, exhibited by two peaks in daily activity, morning peak following sunrise and an evening peak preceding the sunset\(^9\). Much emphasis is given to locomotor activity patterns in migratory and partially migratory birds, probably because of the conspicuous changes (like Zugunruhe) in activity pattern over the year and the unveiled mystery underlying mechanism of migration\(^10,11\). Few studies report locomotor activity pattern in non-migratory low latitude birds\(^11\).

Black headed munia, *Lonchura malacca malacca* is a post-monsoon breeding tropical estrildid finch inhabiting Sri Lanka, Australia and Indian peninsula\(^12\). In nature, it shows seasonality in gonadal stimulation\(^13,14\) and conspicuous gonadal stimulation under a wide range of artificially altered daylengths (3L, 9L or 16L) followed by complete elimination of gonadal regression for a year or more\(^14,15\). The present study sought to observe seasonality in circadian clock characteristics by monitoring daylength driven behaviour of blackheaded munia under long days (14L: 10D), short days (10L: 14D), continuous conditions of dim and bright light and to describe seasonal trend in daily pattern of activity/ rest cycle under natural illumination conditions (NDL).
Materials and Methods
Adult blackheaded munia were captured and placed in an outdoor aviary for acclimatization (Ghaziabad, India: 28.6° N; 77.4° E). In aviary, all birds received natural light and temperature conditions (NDL). Males were distinguished from females through unilateral laparotomy as per the procedure described by Kumar et al. Briefly, gonads (testes or ovary) were located through a small incision made on left flank between the last two ribs using local anesthesia. The incision was stitched and antibacterial ointment was applied. Indoors, birds were housed singly in cages (size 36 × 30 × 30 cm³) in lightproof boxes (size 1 × 1 × 1 m³) provided with light from white compact fluorescent lamp at ~500 lux. Automatic time switches controlled periods of light and darkness. The photoperiodic chambers were well aerated and the temperature inside these chambers did not vary more than 1-2 °C from the ambient temperature. The light intensity was measured at the perch level. Food and water were provided ad libitum, and replenished only during the light phase, except under continuous dim light conditions.

The experiments were performed at the MMH College, Ghaziabad, as per CCS University Meerut- Institutional Ethics Committee guidelines. Following two experiments were performed:

Experiment 1: Daily pattern of locomotor activity under artificial daylength
This was studied under two parts. Part one (experiment 1A) compared locomotor activity of munia under 14L: 10D (LD; lights on- 0800 hrs; lights off- 2200 hrs) and 10L: 14D (SD) by observing the entrainment properties of voluntary activity. In experiment 1B, two groups of birds were held under 12L:12D (light on- 0800 hrs; lights off- 2000 hrs) and 10L: 14D (SD) by observing the entrainment properties of voluntary activity. In experiment 1B, two groups of birds were held under 12L:12D (light on- 0800 hrs; lights off- 2000 hrs) for 14-18days. Thereon, one group was released into constant photoperiodic conditions of continuous dimlight (DD; 2-3 lux at perch level) and other group was released into continuous bright light (LL; >300 lux at perch level) to test whether circadian rhythmicity persists in activity behaviour of munia under the above conditions.

Experiment 2: Seasonal pattern of locomotor activity under natural daylength conditions
Locomotor activity of munia (n=6, n=4 in May; two birds died during the experiment) held in individual activity cages receiving natural illumination (28.6° N) from November 2008 to May 2009 was monitored. The experiment had to be discontinued because of high mortality in birds due to outdoor summer heat at Ghaziabad in May.

Activity recording
To measure individual activity, birds were housed separately in activity cages (see also Malik et al.17). Each activity cage had two perches and an infrared sensor system (Napoleon Pet, Maximum 8, India) mounted in front to detect the movement of bird within cage. Thus, general 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) that stored individual perch-hopping activity in separate channels. Activity records (actograms) were obtained by plotting successive days beneath one another and double plotted for visual facilitation. Actogram were quantified into total activity/half hour bin for selected duration of experiment and averaged to get daily activity profile for each bird and subsequently mean±SE of the group was calculated.

All data on activity are plotted as mean±SE, while the actograms given are for representative birds. One-way analysis of variance with repeated measures (one-way RM ANOVA) was performed to determine the significance in changes inactivity pattern between same group at different time points, while two groups at the same time point were compared using the Student’s unpaired t-test. Significance was taken at P < 0.05. Statistical analyses were performed using Prism Graph Pad software.

Results
Daily pattern of locomotor activity rhythms under artificial daylength—Figure 1 presents daily activity pattern through double plotted actograms (left panel) and daily activity profiles obtained by plotting average hourly activity. In general, the birds were day active with greater activity in first half of the day (Figs 1a and b). The onset of activity coincided with the lights-on under LD whereas the onset of activity preceded the lights-on (phase difference- 0.83±0.12 h) under SD. In particular, average hourly activity during daytime was higher in SD than that in LD (Fig. 1c). Figure 1d shows that the morning activity, M (total activity during 2 h after lights on) under LD was significantly more than that under SD. Also, the evening activity (E, total activity during 2 h before
lights off) was compromised under LD than in SD ($P<0.05$, Student’s unpaired $t$-test). Total daytime and total nighttime activity varied significantly between the LD and SD groups ($F_{1,12} = 16.82$, $P < 0.01$, one-way RM ANOVA) (Fig. 1c), but the two groups did not differ ($P = 0.9142$ at df =46, unpaired $t$-test) in their daily activity profiles.

Figure 2 shows the actograms and daily activity profile of groups of munia exposed to 12L:12D, followed by their release into constant photoperiodic conditions. Activity of birds synchronized to equinox daylength, with significantly higher morning activity, M (total activity in 2 h after lights on) than evening activity, E (unpaired $t$-test, $P< 0.0001$, $t_{78}=6.551$, value compared for 10 days for 4 birds). Daily activity decreased in the group of birds exposed to constant dim light (DD; 2-3 lux at perch level). Three of the four birds free-ran with a period ($\tau$) of 24.83 ± 0.06 h,
while one bird free-ran with a period (τ) of 25.9 h. Munia under LL exhibited an increase in overall activity, but did not reveal any consistent trend through time series analysis, thus exhibiting arrhythmia under constant LL.

Seasonal pattern of locomotor activity behavior under natural daylength conditions—Figure 3 shows activity rhythms of munia held in individual activity cages receiving natural illumination (NDL) from November 2008 to May 2009, thus covering winter, spring and summer seasons in the year. NDL data are plotted with 0600 hour morning time as clock hour 0, for visual facilitation. There is a clear seasonal pattern in daily activity profile from November to May.
Daily activity entrained to seasonal changes in daylength with a conspicuous morning peak and a less conspicuous the evening peak as the daylength started increasing in January; persisted in February and disappears after March (Figs. 3a, b−vii). There was a decrease in daytime activity from April onwards, could be due to increasing summer heat, as already stated.

Discussion
The present study had a straightforward protocol to describe seasonal trend in daily pattern of activity/rest cycle under natural illumination conditions. Some visible trends while graphing of data did not reveal significant difference during statistical analysis. One reason for this lack of statistical differentiation was due to the low number of study animals and decreased activity in captive birds. Funnel and Munro observed dampening of activity of captive birds for two consecutive years during activity recording of the partially migratory Tasmanian silvereye, Zosterops l. lateralis, and the non-migratory mainland silvereye, Z. l. familiaris. The blackheaded munia did not exhibit any nocturnal activity like the Tasmanian silvereye.

Activity pattern in munia exhibited phenotypic plasticity over the seasons, although with a single peak of activity daily. Comparison of activity behavior indicated that morning activity, M was significantly higher than evening activity, E in LD (P< 0.01, t= 6.92; paired t-test) while there was no significant difference in M and E activity under SD (Figs 1 b and d), suggesting a possibility that reduced light duration imposes a constraint of activity hours in munia or induces a change in coupling of component M and E oscillators. SD birds exhibited greater total daytime activity than LD birds in agreement with Helm’s suggestion, but munia exhibited absence of Zugunruhe (night restlessness). Also, activity patterns of munia differed under natural and artificial rectangular light dark cycles. In NDL, there was no anticipatory activity in December (daylength-10.3 hours) while anticipatory activity to lights on was observed in artificial 10L: 14D.

A free-running activity rhythm under constant dim light DD, suggested persistence of circadian period in blackheaded munia. Bright light induced arrhythmia although the possibility of masking by the constant bright light schedule could not be ruled out. Yamada suggested that bright LL might be acting on the core of the circadian system, rather than on the pathway between the pacemaker and observed rhythms in pigeons.

Blackheaded munia may be an interesting species to address the hypothesis that the daily activity-time and the period (τ) of the circadian activity rhythm are affected both by external variables (daylength) and also by internal changes in the hormonal balance related to the annual cycle of reproduction because it shows gonadal stimulation under 9L and 15L followed by complete elimination of gonadal regression under long days. In munia, under NDL annual changes in daylength affected changes in daily pattern of activity rest cycle and amount of activity but more fundamental characteristics like period of circadian rhythm and phase angles were not affected. Nevertheless, this is first detailed study to date on the daily and seasonal activity patterns of blackheaded munia in captivity.

A simultaneous recording of the activity pattern in different light conditions (viz. LD, SD, LL and DD) suggested that the behavioral responses in the blackheaded munia are mediated by circadian system.

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