



Physiology of Temporal Birds and environmental Effect: A Study

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ABSTRACT

Avian behaviour and physiology are embedded in time at many levels of biological organization. The seasonal changes in day and night cycle influence daily pattern of locomotor activity which is linked with exploration and foraging. Also, the body temperature is influenced by these behaviours. Therefore, in the present study we have tried to demonstrate the photoperiod dependent circadian modulation in locomotor activity, food intake and temperature rhythms and to analyze the change in their phase relation as a consequence of changing photoperiods. Spotted munia (*Lonchura punctulata*) (n=6 per group) were exposed to different photoperiods; 4L:20D, 8L:16D, 12L:12D, 16L:8D, 20L:4D and LL. For a period of 4 weeks, we observed diverse activity patterns in different photoperiods' viz. unimodal (4L, 8L and 20L groups), bimodal (12L and 16L groups) and amodal (LL group). However the acrophase for activity did not differ between photoperiods except in LL, was delayed. There was no significant difference in the total activity counts in all the photoperiods. The body temperature lost its rhythmicity only in 4L group. The food intake pattern was similar to locomotor activity and was rhythmic in all groups except LL. Phase relation in the food intake, activity and body temperature also drifted apart with each other with increasing photoperiods. The results suggest that daily activities in locomotion and food intake along with the temperature shows photoperiod specific changes.

Keywords: Photoperiod, spotted munia, activity, food intake,

INTRODUCTION

Life on earth is exposed to gradually changing environmental conditions, more dramatic at higher latitudes than at equator and the animals take cue from these changes and programme their reproduction and associated phenologies (Kumar, 2017, Hahn et al., 1997, Sharp, 2005). The survivorship of birds get enhanced by phasing their daily activities such that they occur at an optimal time of the day involving an endogenous circadian (circa = about; dian = day) timing system and in synchrony with the periodicity of the environment (e.g. light–dark cycle), a clock-controlled activity is expressed as a daily rhythm at physiological and behavioural levels (Aschoff 1982, Aschoff 1981, Gwinner and Brandstatter, 2001, Budki et al., 2009). As vertebrates exhibit rhythms with frequencies ranging from milliseconds to months in their many behaviours and life processes which are parallel, but are not the reactions of, the environmental cycles (e.g. light-dark cycle). They are generated by self-sustained endogenous clocks, and they continue to express as overt rhythms even in the absence of an input cue from the environment (Rani et al., 2006, Kumar and Singh, 2006). They set up a local time with respect to environmental cycles, and run at the same frequency at different temperatures (example of temperature compensated biological clocks). Hence, they ensure the timekeeping in an organism such that it can optimally survive in its chronological environment (Kumar and Singh, 2006). The environmental cues for example proximate cues (photoperiod) exerts a selection pressure either by restricting an activity to a particular time of the year and they are useful in those environments which show predictable seasonal changes (Gwinner, 1986). Contrarily, the tropical breeders, which live in almost constant photoperiodic environments, have to rely on endogenous circannual clock that allows advance preparation for confirming better endurance (Kumar, 2017, Gwinner, 1992). On the other hand ultimate factors (food, temperature, rainfall etc.) trigger the annual cycles by affecting the physiological processes (Baker, 1938). As an effect birds initiate physiological and morphological changes well in advance enough to be completed on time. Ultimate factors play a role in synchronizing the seasonal events but are not directly related to fitness (for example breeding success or good molting conditions). The temperature and food have more direct links to fitness and, therefore, may combine proximate and ultimate aspects. In vertebrates, the timing system controlling the most biological rhythms is multifaceted and formed of multiple pacemakers. In mammals, the cells in the SCN regulate most of the rhythmic functions that occur in a circadian manner (Bell-Pedersen et al. 2005, Panda et al. 2002). Birds are equipped with highly complex and diverse clock system as autonomous clocks in birds exist in at least three structures: the retina, the pineal gland and the hypothalamus. These appear to interact and form a central clocking system which is plastic in nature, and the existence of multiple clocks helps them to stabilize each other in order to produce a highly stable circadian output (Underwood et al. 1994). Circadian organization is to be adaptive, it is logical to assume that the avian Central clock system will be diverse at the species level, since birds inhabit varied environments and latitudes having different photoperiods at the same time (Martin et al. 2020). Circadian melatonin rhythm (has almost opposite phase relationship with body temperature) varies among species inhabiting the same latitude (Van't Hof et al. 1998). Also the species which share the same environment and latitude may contain different temporal plans for its survival. There is a wide species differences in the features of the circadian rhythmicity among different birds and literature on birds seem to indicate the complexity of the

circadian organisation found in nonmammalian vertebrates (Dixit and Singh, 2016). Birds anticipate the changes in their environment on daily basis to optimise the resource utilization and maximise their reproductive fitness (adaptations in daily activities; which are readouts of time-of-day which are under circadian (circa = about; dian = day) control (Kumar, 2017, Agarwal N; Thesis, 2016, Hastings et al. 2003). Environmental cues (photoperiod, food and temperature etc.) play an important role in the regulation of an animal's physiology and behaviour for example locomotor activity, body temperature and food intake in birds (Jia-Qi Wang et al. 2016). Studies also suggest that avian behavior and physiology gets affected by shorter and longer photoperiods making changes in the duration of animals' activity. Birds maintain a high and constant body temperature (with daily torpor) and this enables them to exploit not only tropical, temperate but polar habitats too (Reyes-Arriagada, 2015, Yahav, 2015). Furthermore, daily cycle in body temperature is closely related to photoperiod and is weakly circadian (entrained by dusk rather than dawn (Dawson, 2017). In this study we wanted to see the effect of exposure photoperiod of different latitudes (either short or long photoperiods) on circadian or daily modulation in behavior (locomotor activity) and physiology (body temperature and food intake) in spotted munia (*Lonchura punctulata*). We also assessed duration and pattern of rest (period of inactivity) in different photoperiods and its relationship with physiological rhythms like body temperature and food intake.

MATERIAL AND METHODS

The birds; spotted munia (*Lonchura punctulata*), procured and placed in an outdoor aviary for acclimatization (two weeks) where they received natural day length (NDL) and temperature conditions, then transferred into activity facility for the experiment. On 24th November 2015, birds (N=6; adult birds per group i.e. 4L: 20D, 8L: 16D, 12L: 12D, 16L: 8D, 20L: 4D, LL) placed into individual activity recording cages (Cage size 45×30×40 and chamber size 56×56×56). The light intensity during the day and night was 220-270 lux and 0 lux respectively (15 watts, Bajaj). The birds received ad libitum food (*Setaria italica* with *Oryza sativa*), and water throughout the experiment (Singh et al., 2010). The temperature was 22±1 °C during the entire experiment and the experiment was performed at University of Lucknow, Lucknow as per the approval of the Institutional Animal Ethics Committee. After the termination of the experiment, birds were released in the nature. The experiment compared 24h changes in the pattern of food intake, body temperature and locomotor activity and rest of the birds under different photoperiods (4L:20D, 8L:16D, 12L:12D, 16L:8D, 20L:4D and LL). The total activity counts (mean ± SEM) of 15 days (during which the observations were taken) were plotted. Body temperatures were recorded with the help of Thermo scan (Quick shot infra-red thermometer, model EXP-01B) on zeitgeber time (ZT) 2, 5, 8, 11, 14, 17, 20 and 23 from the furcular region for two days and the mean ± SEM of two days were plotted. Food intake of every three hours was measured by spreading the paper sheet under the cage at the same ZT as body temperature i.e. ZT 2, 5, 8, 11, 14, 17, 20 and 23 for two days and then two days mean ± SEM were plotted. We also calculated the acrophase for body temperature and food intake by doing the Cosiner analysis (and manually for locomotor activity). We also calculated rest/ hour and ratio of rest/activity in all the photoperiods to check the amount of rest taken by the birds in all the photoperiods at every hour of the day.

Photoperiod specific changes in activity behavior and body temperature of birds

Photoperiod specific patterns of daily activity rhythms were observed in this study. Birds, when exposed to 4L:20D, 8L:16D, 12L:12D, 16L:8D, 20L:4D and LL, showed entrainment of their locomotor activity rhythm and their activity was found confined mainly during the light phase. However in 4L:20D the activity dispersed more than 4 hours of available light plausibly because of ultra-short photoperiod. Ultra short photoperiods are often responsible for the anticipatory activity in birds (Sean and Brian, 2014). Birds develop bimodality in activity patterns after 12L:12D photoperiod only which indicates that the high evening activity may be photoperiod dependant and if it is so, the evening activity will either disappear and fade away in photoperiod below 12L:12D photoperiod. We observed that the evening activity peak was sensitive for the change in photoperiod as in photoperiods higher than 12L:12D, it's amplitude lowered and in photoperiods larger than 16L:8D, it was almost negligible. Evening burst of activity is a characteristic feature in many animals from flies to vertebrates (Aschoff, 1966, Grima B et al. 2004 and Santiago-Quesada et al. 2021). On one hand, as the photoperiod increased the activity rhythm showed changes accordingly and in constant light (LL) it lost its most of the amplitude and became somewhat flat, body temperature on the other hand, showed prominent rhythmic features and was found rhythmic in constant light conditions, signifying its role in maintaining body homeostasis. In our study, the body temperature mainly remained rhythmic except in (4L:20D photoperiod) with photoperiod dependant changes in its acrophase. Animals having hypothermia reduces energy expenditure and increases the probabilities of surviving winters (Maddocks and Geiser, 1997, Clark and Dukas, 2000), and birds by reduced food intake show a lower daily values in body temperature (Thouzeau et al. 1999). In another study, Van der Leest et al. 2009, on mice that short photoperiod (8L:16D) lowers the daily levels of electrical activity more in comparison to a long photoperiod (16L:8D) indicating the role of shorter photoperiod on animal body temperature. The daily cycle in melatonin has been found implicated in the control of cycles in body temperature and some young starlings do not develop fully until after they become homoeothermic after getting adult (Dawson and Van't Hof, 2002, Binkley et al. 1971, Oshima et al. 1989, Underwood, 1994, Dawson and King, 1994). Alistair Dawson, 2017 reported in European starlings, that avian body temperatures are weekly circadian and gets affected by a change in photoperiod (Dawson, 2017) although further studies will use this information to assess the involvement of energetic (for example food availability and ambient temperature) on photoperiodic responses. Food intake patterns surprised us as, first of all, they showed rhythmicity in all the photoperiod except in constant light (LL). Moreover, the amount of food intake was interestingly was not hand in hand as the activity patterns in all the photoperiods. It showed patterns like activity rhythm and bimodality developed with an increase in photoperiod slowly. In 4L:20D and 8L:16D, it showed antiphase in the context that, activity was at its peak during morning while the food intake was maximum around evening activity peak which shows that the birds were remain very much aware of the onset of dark to save itself from the food scarcity. In larger photoperiods, the rhythm of food intake also flattened although there was no significant difference was observed in total food intake in all the photoperiods, possibly the birds were intelligent enough to distribute its food resources during different times of a day (Santiago-Quesada et al. 2021). To sum up, this experiment investigated the physiological flexibility of spotted munia under a wide range of photoperiodic

schedules that how it adapts to changing photoperiodic conditions. No bird casualty occurred in this experiment which is an indicative of high physiological flexibility of the birds under the study.

Effect of heat stress on birds

The rising global temperatures leading to global warming, has adversely affected the stability of prevailing environment along with changing ambient temperature and humidity (IPCC, 2007, Sakercioglu et al. 2012). This change has enhanced the magnitude and frequency of extreme weather events, producing hot days in the form of heat stress which cause negative effects on organism's metabolism, protein structure and other physiological processes (Karl et al. 2011, Teves and Henikof, 2011). The majority of animals have repair mechanisms to neutralize these negative effects of heat stress, but they are generally only effective at 'normal' temperature regimes (Rodriguez-Trelles et al. 2013). Extreme hot events like heat waves, often occur multiple times during the life span of an organism (Zhang et al., 2015b). Thus, the effect of hot days on organism should depend on the delicate balance of damage versus repair periods (Zhang et al., 2015b, Sakercioglu et al. 2012, Perkins and Alexander, 2013). Every year, high environmental temperatures attribute to mass die-offs of birds, as the combination of summer with extreme climatic events encourage more hot days which has huge impact on the predicted movement ranges of animals like birds and destruction of their population (Doerr and Santín, 2016). It is expected that the frequency of hot days in the form of heatwaves and wild forest fires will increase in many more countries as tropical forest fires are now more frequent, severe and large (Cochrane, 2003, AnneSophie, 2017). Short periods of extreme temperatures separated by sufficiently long periods of normal temperatures may allow organisms to recover fast but in contrast, if hot periods last multiple days without normal days for recovery and repair, the negative effects may rapidly accumulate and result in irreparable damages to the organisms or even death (Bailey and van de Pol, 2016). Hot days in summers in India are preceded by the cool winters and succeeded by the rainy monsoon season, but it becomes even more challenging when the hot spells of May continue to persist in the rainy season, leading to delay or failure of the monsoon winds that carries moisture-laden air (i.e., rainfall) to the Indian mainland (Webster et al., 1998, Panda et al., 2017). Globally, studies have shown that heat stress in the form of hot days are associated with droughts and moisture shortfalls including the European, Russian, Texas and California droughts and heatwaves (AghaKouchak et al. 2014, Hauser et al. 2016, Murari et al. 2016). India too witnessed this situation wherein draught and heatwaves coexisted resulting into crop failures, water storage loss and ecological disturbances (Panda and Wahr, 2016). Reports confirm that heat stress induces various pathophysiological responses such as metabolic disorders, decreased food consumption and increased blood glucocorticoid and other biochemical levels (Mohammad-Borhan et al. 2015, Whitefield et al. 2015) and it can alter the timing of both circadian and seasonal phenologies linked with different life history stages in birds (Singh et al. 2012). Studies reported that time of the day and niche specific patterns of activity-rest, food intake and various physiological and biochemical processes play a critical role in avoidance of hotter days (Xie et al. 2018, Cassone, 2017). As hot days having temperature upto 45-50 °C were experienced in large parts of northwest, central and east India every year from past some years (India Meteorological Department; IMD), thus, prevalence of heatwave generated our interest in understanding how the birds respond to this type of climatic havoc. Therefore, in the present study, we chose a resident passerine finch; spotted munia (*Lonchura punctulata*) to investigate the possible effect of simulated heat wave on some of its behavioral, physiological, hematological and biochemical parameters.

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