J Endocrinol Reprod 19 (2015) 2 : 69 - 80,

Online ISSN: 0971-913X
Print ISSN: 2455–1244
DOI: 10.18311/jer/2016/7654

Original Article

Thyroid gland in regulation of annual reproduction and oxidative metabolism of a tropical bird, *Perdicula asiatica*: Role of melatonin and environmental factors

V. K. Verma, S. K. Yadav, C. Haldar* and R. K. Tripathi

Pineal Research Laboratory, Department of Zoology,
Institute of Science, Banaras Hindu University,
Varanasi - 221005, Uttar Pradesh, India

Abstract

As a transducer of the environmental factors the pineal gland, together with the metabolically active thyroid gland, plays a major role in control of reproduction during different times of the year, in response to the changing environmental conditions of the tropical zone unlike in the temperate zone. Our avian model *P. asiatica* is a long day breeder. It is reproductively active during summer and quiescent during winter months. In this study we investigated the role of thyroid gland super-imposed by melatonin in the regulation of the annual male reproduction in this bird. The metabolically active thyroid gland presented a functional parallelism with the testicular activity suggesting that thyroid hormone is essential for reproductive activity and related metabolic energy for avian species. Our data also suggest an inhibitory effect of melatonin on thyroid gland function [weight, thyroxine (T3/ T4) level and thymidine kinase activity, THK] both during active and inactive phases of the reproductive cycle. However, this inhibitory effect was less evident during the active phase. The low level of melatonin during the reproductively active phase might be due to long days of summer which prevented the birds from being hyperthyroidic. Therefore, we suggest that the level of melatonin serves as a physiological check to control the seasonal reproductive activities of gonads and thyroid which synergistically play most important physiological roles in energy metabolism of these seasonally breeding bird *P. asiatica.*

Key words: Bird, Environmental Factors, Melatonin, Reproduction, Thyroid

Introduction

It has been proposed that avian pineal gland translates photoperiodic information and acts as interphase between photoperiod and other endocrine glands and some metabolic functions (Wood and Loudon, 2014). Changes in environmental light modulate melatonin level to promote annual variation in gonadal activity and *vice versa* (Sudhakumari et al., 2001; Garcia et al., 2003). Melatonin is considered as the most physiologically active indole derivative in birds affecting locomotor activity, reproduction, renal functions, immune and metabolic system etc. (Gwinner, 1989; Reiter and Maestroni, 1999; Pevet, 2000; Csernus and Mess, 2003).

In avian species thyroid hormones play a major role in regulation of the reproductive function, breeding cycle, migration, oxidative metabolism, growth and development, and electrolyte and water metabolism (Gupta and Thapliyal, 1991; Shinomiya et al., 2014). Thyroid hormone also helps birds in continuous adjustments of physiological and morphological functions in relation to the ever changing environment (Todini, 2007). There are reports showing that thyroid hormones play major roles in regulation of energy metabolism and reproduction of mammals (Francis, 2014).There is lack of information on detailed account of annual variations in the levels of the thyroid hormone, and reproductive status (testis weight/ testosterone level) in correlation with melatonin level in tropical birds.

In most of the vertebrates the thyroid hormone production is very high during summer months when day is long, and pineal weight and melatonin level are very low and *vice versa* during

***Correspondence to be addressed to:** Dr. Chandana Haldar, Ph.D.; Email:chaldar2001@yahoo.com

winter months (Johansson et al., 2001; Morera and Abreu, 2006). Melatonin has been reported to influence thyroid activity in mammals and *vice versa* (Ozturk et al., 2000; Singh et al., 2006). The level of thyroid hormone is inhibited by administration of melatonin and increased following pinealectomy in mammals (Vaughan and Pruitt, 1985; Wajs et al., 1992; Ozturk et al., 2000) and also in temperate zone birds (Prakash et al., 1998). However, there is lack of information about the effects of melatonin and thyroid hormones in any avian species on oxidative stress as well as gonadal and thyroid function. Therefore, the objective of present study was to find the relation between annual variation in the pineal gland activity (in terms of glandular weight and melatonin level) and thyroid gland status (glandular weight, peripheral T3/T4 levels and THK activity) with that of thyroidal free radical load in a seasonally breeding tropical bird that confronts drastic environmental (photoperiod, temperature, humidity seasonal diseases, lack of food and shelter) challenges.

Materials and Methods

The experiments were conducted with adult male bird *Perdicula asiatica* (body mass 35–40 g) for two consecutive years and means of the data are presented. The birds were collected from the vicinity of Varanasi (Lat. 25°, 18′ N, Long. 83°, 01′ E) during the first week of each month and acclimatized for two weeks in an open-air fenced aviary exposed to the ambient environmental conditions. The birds were fed with millet seeds (*Pennisetum typhoideum*) and other seasonal grains and water *ad libitum*. All the experiments were performed in accordance with institutional practice and within the framework of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines.

Sample collection

The male birds were selected randomly (n=7), and sacrificed by decapitation in red light under mild anesthesia during the last week of each month. One night (at 22.00 h) prior to sacrifice, birds were bled through pectoral vein for radio immunoassay of melatonin and then sacrificed on the following day (at 11.00 h). The daytime-collected blood was kept in heparinized tubes, and plasma was separated and stored at −20 °C for radioimmunoassay of melatonin and testosterone, and ELISA of T3/T4. Pineal gland, testis and thyroid were dissected out on ice, weighed in an electronicbalance and then processed for histology and various assays.

Hormonal analysis

The plasma content of melatonin and testosterone were determined using the modified radioimmunoassay method of Rollag and Niswender (1976) for melatonin, and RIA kit from Immunochemical Corporation, Carson, USA, for testosterone. The validation of radioimmunoassay was performed as described earlier (Haldar and Rai, 1997; Sudhakumari et al., 2001). The intra- and inter- assay variations for melatonin were 9 and 15% and for testosterone 4.5 and 5.6%, respectively. The sensitivity for melatonin RIA was 18–20 pg/mL and for testosterone RIA was 6 pg/mL. The recovery of melatonin and testosterone RIA was 92% and 95%, respectively, and the plasma level of total T3 and total T4 were measured adopting radioimmunoassay using kits (RIAK4/4A for T3 and RIAK5/5A for T4) procured from the board of radiation and isotope technology (BRIT) Mumbai. The RIA of T3 and T4 were conducted following manufacturer's protocol with slight modification. The intra- and inter- assay variations were found to be on an average less than 3.5% and 6.5% for T3 and T4, respectively, and the recovery was 95%. The sensitivity for T3/T4 RIA was 0.5 μg/ dL.

Blood leukocyte, percent lymphocyte and heterophil counts

Blood was collected in 1 mL heparinized syringe for total leukocyte count (TLC) in Neubauer's counting chamber (Paul Marienfeld GmBH & Co., KG, Lauda-Königshofen, Germany).

Lymphocyte count (no. /mm^3) was determined from the total and differential leukocyte counts, using the following formula:

$$\% \text{ Lymphocyte count} = \frac{\text{TLC} \times \text{Lymphocyte percentage}}{100}$$

Heterophil/lymphocyte (H/L) ratio was counted from blood smear following the method of Ots et al. (1998) under the microscope (Nikon, Kawasaki, Japan). All the cell counts within a set were done by a single observer. For each set, five slides were counted at least three times.

Lipid peroxidation (LPO) assay by estimation of thiobarbituric acid reactive substances (TBARS) level

After sacrifice of birds, the thyroid was dissected out on a sterile watch glass placed in ice box, cleaned from adherent tissues and processed immediately for estimation of lipid peroxidation. Thyroids of experimental birds were weighed and homogenized in a ten-fold excess of 20 mMTris–HCl buffer (pH 7.4) and the 10% homogenates were centrifuged for 15 min at 3000 x g at 4 °C. The supernatant was subjected to thiobarbituric acid (TBA) assay by mixing with 8.1% sodium dodecyl sulfate (SDS), 20% acetic acid, 0.8% TBA and then digested for 1 h at 95 °C. The reaction mixture was immediately cooled in running water, vigorously shaken with 2.5 mL of n-butanol and pyridine reagent (15:1) and centrifuged for 10 min at 1500 x g (Ohkawa et al., 1978). The absorbance of the upper phase was measured at 534 nm. Total thiobarbituric acid reactive substances (TBARS) were expressed as malondialdehyde (MDA; nM/g tissue weight) taking 1, 1, 1, 1-tetraethoxy propane (TEP) as the standard. The standard curve was calibrated using 10 nM TEP.

Superoxide dismutase activity

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed following the method of Das et al. (2000). Just after sacrifice, 10% homogenate of thyroid was prepared in 150 mM phosphate buffered saline (PBS, pH 7.4) and centrifuged for 30 min at 12,000 x g at 4 °C. The supernatant was again centrifuged for 60 min at 12,000 x g at 4 °C and then processed for determination of enzymic activity based on a modified spectrophotometric method using nitrite formation by superoxide radicals. A 0.5 mL of the homogenate was added to 1.4 mL of reaction mixture comprised of 50 mM phosphate buffer (pH 7.4), 20 mM L-methionine, 1% (v/v) Triton X-100, 10 mM hydroxylamine hydrochloride, 50 mM ethylene diaminetetraacetic acid (EDTA) followed by a brief pre-incubation at 37 °C for 5 min. Next, 0.8 mL of riboflavin was added to all samples along with a control containing buffer instead of sample and then exposed to two 20W fluorescent lamps fitted parallel to each other in an aluminum foil-coated wooden box. After 10 min exposure, 1 mL of Greiss reagent was added and absorbance of the color was measured at 543 nm. One unit of enzyme activity is defined as the amount of SOD inhibiting 50% of nitrite formation under the assay condition.

Thymidine kinase assay

The thyroid lobes of birds were incubated for 4 hr in RPMI 1640 medium (Gibco), with an addition of 20 mM of Hepes buffer, 15% FCS, penicillin (200IU/ml), and streptomycin (19µg/ml). After the incubation, the thyroid lobes were homogenized in the medium (25mMTris-HCl buffer, 25 mMKCl and 5 mM $MgCl_2$Ph 7.4, temp. 0°C). Following centrifugation (10000 x g for 20 min), the post-mitochondrial fraction (70µl) was incubated for 30 min (37°C) with addition of labeled thymidine [2-^{14}C] dThd. The reaction was terminated by subjecting the tubes to boiling water bath (100°C, 2 minutes). After de-proteination (centrifugation for three minutes) the aliquots of the supernatant were placed on the Whatman DE81 chromatography paper. The reaction products (d-TMP and d-Thd) were separated by ascending chromatography at room temperature in a solvent of 5mM of ammonium formate (pH 5.6). The chromatograms were dried, the radioactive spots corresponding to d-TMP and d-Thd were cut out and placed in counting vials. Radioactivity was measured in a liquid scintillation counter. The enzyme activity was expressed in cpm /min/ mg of protein.

Statistical analysis

Statistical analysis of the data was performed with one-way ANOVA with the help of SPSS Statistics 17.0 software. The data were presented as means ± SEM. The difference was considered significant when $p<0.05$.

Results

Annual variation in climatic factor

The annual climatic variations, shown in figure 1 for one year 2004-2005, were recorded at Varanasi (Lat.25 18'N; Long. 83 '1E) from the Meteorological Department of the University. The maximum temperature, 45 °C, was recorded in June, and the minimum,6°C,was recorded in January. The maximum day length, 14L: 10D, was recorded in of June and the minimum, 10L: 14D, was recorded in December. The maximum humidity due to rainfall was recorded in January, July-August and the minimum was recorded in June (Fig.1a).

Annual variation in pineal and testis weight

There was a significant increase in pineal weight during July to December ($p<0.01$) compared to January. The maximum pineal weight was recordedin December (5.5 ± 0.28 mg/100 g bwt) and the minimum was recorded in June (0.8±0.023 mg/100 g bwt). On the other hand, a significant decrease ($p< 0.01$) in testis weight was noted in November and December compared to June. The testis weight was maximum (110.5 ± 0.80 mg/100 g bwt) in June and the minimum testis weight was recorded in January (8.8 ± 0.38 mg/100 g bwt) (Fig.1b).

Annual variation in plasma melatonin and testosterone levels

There was a significant increase in plasma melatonin level during July to December ($p<0.01$) when compared to January. The highest peripheral melatonin level was noted in December (182.5±0.78 pg/ml) and lowest melatonin level was noted in June (31±0.40 pg/mL). There was a significant increase ($p< 0.01$) in testosterone level in March and April when compared to June. The testosterone level was the highest (25 ± 0.85 pg/mL) in April and the lowest testosterone level was noted in the December (6.5 ± 0.64 pg/mL) (Fig. 2a).

Annual variation in T3/T4 level and thyroid weight

There was a significant annual variation in T3/T4 levels and thyroid weight during March to

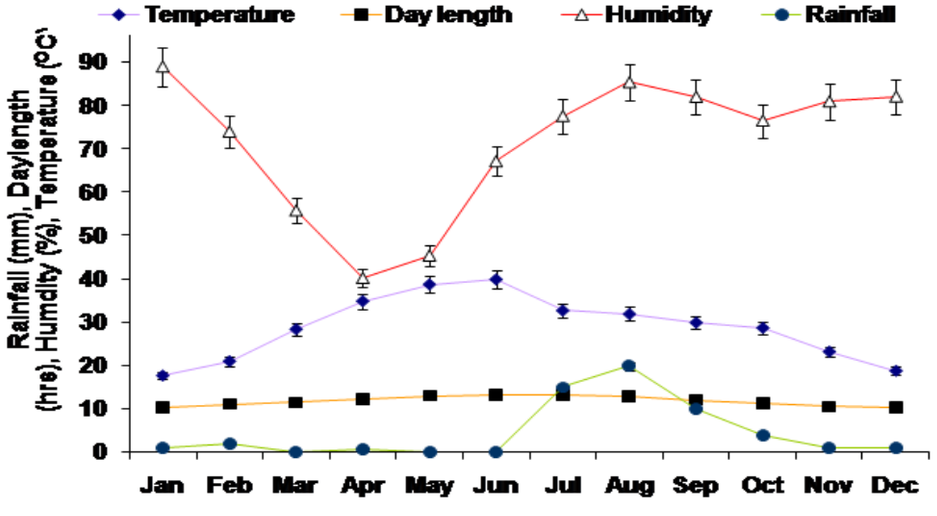

Fig. 1a. Climatic variation during the year 2004-2005 at Varanasi (Lat 25o 18`N; Longitude 83o 01` E.).

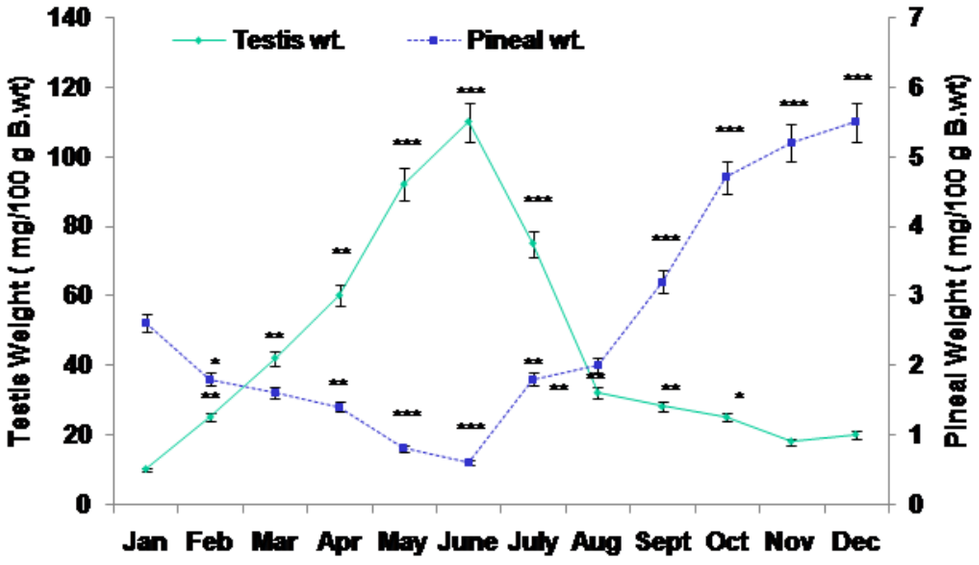

Fig. 1b. Annual variation in pineal weight and testis weight of *P. asiatica*. Data of each point represents mean±SEM; N=7. Vertical bar on each point represents standard error.*p<0.05, **p<0.01, ***p<0.001. The data for January were treated as control and compared with other months.

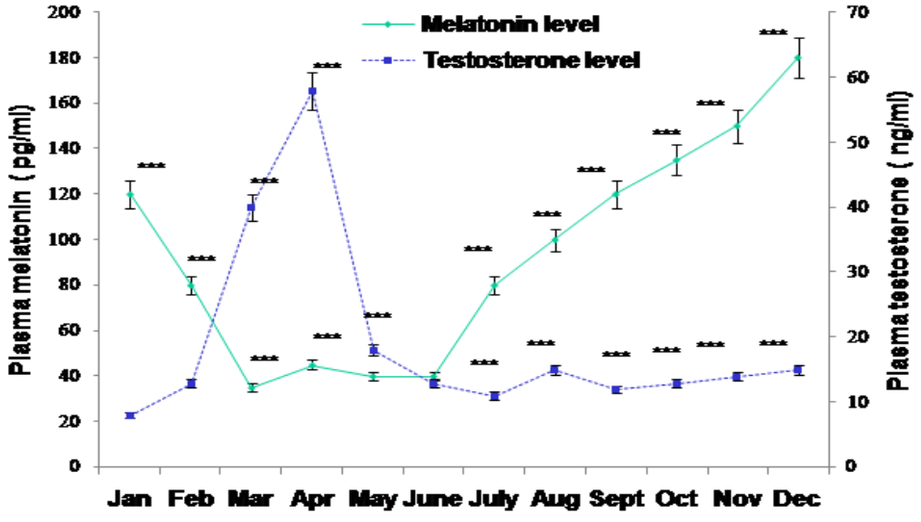

Fig. 2a. Annual variation of plasma melatonin and plasma testosterone levels of *P. asiatica*. Data at each point represents mean± SEM; N=7. Vertical bar on each point represents standard error. *p<0.05, **p<0.01, ***p<0.001The data for January were treated as control and compared with other months.

July (p< 0.05) when compared to January. An increasing trend in thyroid weight and T3/T4 levels was found during March to August, being maximum (2.85±0.082 mg/100g bwt, 108.5±0.68 ng/mL, 124.5±0.62 ng/mL) in June/July. A decreasing trend in thyroid weight and T3/T4 levels was observed during October to January, being minimum in January (1.56±0.043 mg/100g bwt, 94.5± 0.53 ng/ml, 101.5± 0.32 ng/mL) (Fig.2b).

Annual variation in thymidine uptake

There was a significant increase in thymidine uptake during February to September (p<0.01) when compared toJanuary. The minimum thymidine uptake (3811±76 cpm/min/mg of protein) was observed during December. An increasing trend in thymidine uptake was noted from February onwards up to June, where as the maximum value of thymidine uptake (22153 ±

285 cpm/min/mg of protein) was noted in June (Fig.3a).

Annual variation in SOD activity and MDA level in thyroid

There was a significant increase in SOD activity during October to February ($p<0.01$) when compared to January. The maximum SOD activity was found in December (4.11± 0.12 U/g tissue wt). The minimum SOD activity was noted during June to September (0.735 ±0.011U/g tissue wt) and it increased from October onwards up to December. On the other hand a significant increase ($p<0.01$) in MDA level was found from March to July when compared to January.Minimum MDA level (9.5± 0.66nM/g tissue wt) was found during December and maximum MDA level (43.5 ±0.85nM/g tissue wt) was found in June and decreased from August onwards upto December (Fig.3b).

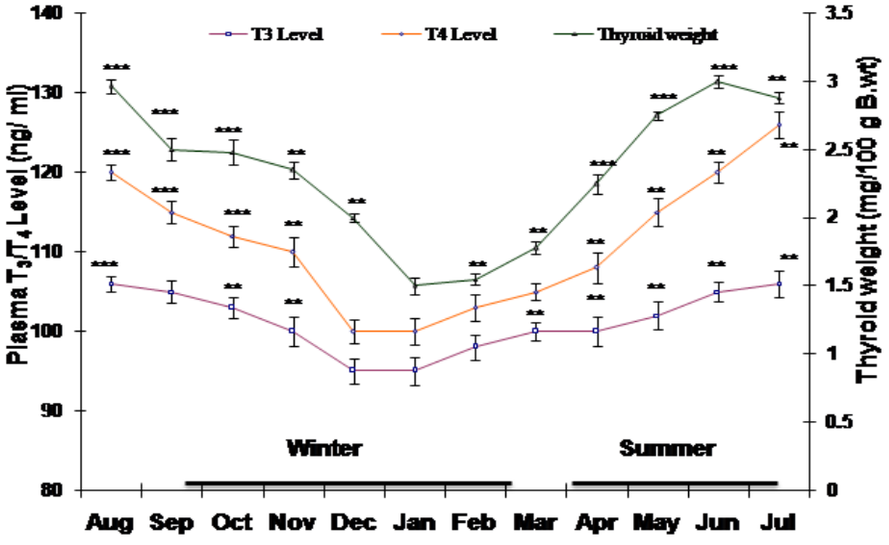

Fig. 2b. Annual variation in the thyroid gland activity (thyroid gland weight and peripheral T3 and T4 level) in *P. asiatica*. Data at each point represents mean±SEM; N=7. Vertical bar on each point represents standard error. *p<0.05, **p<0.01, ***p<0.001The data for January were treated as control and compared with other months.

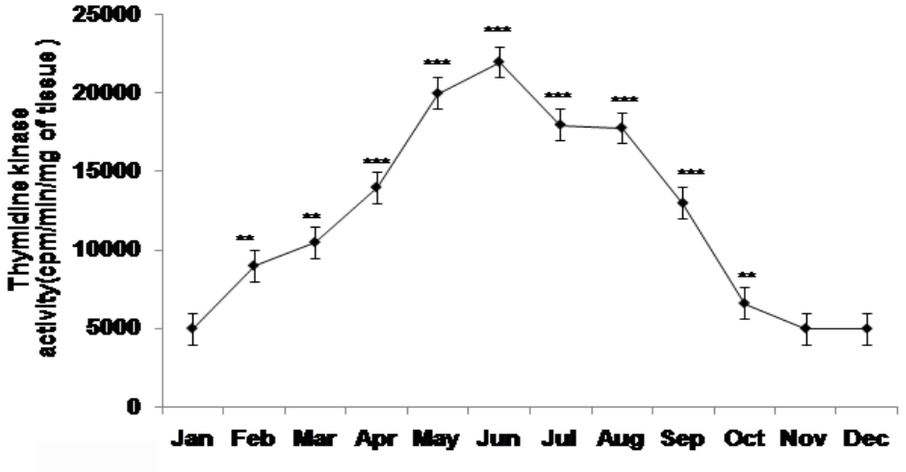

Fig. 3a. Annual variation in thymidine kinase activity of *P. asiatica*. Data at each point represents mean± SEM; N=7. *p<0.05, **p<0.01, ***p<0.001.The data for January were treated as control and compared with other months.

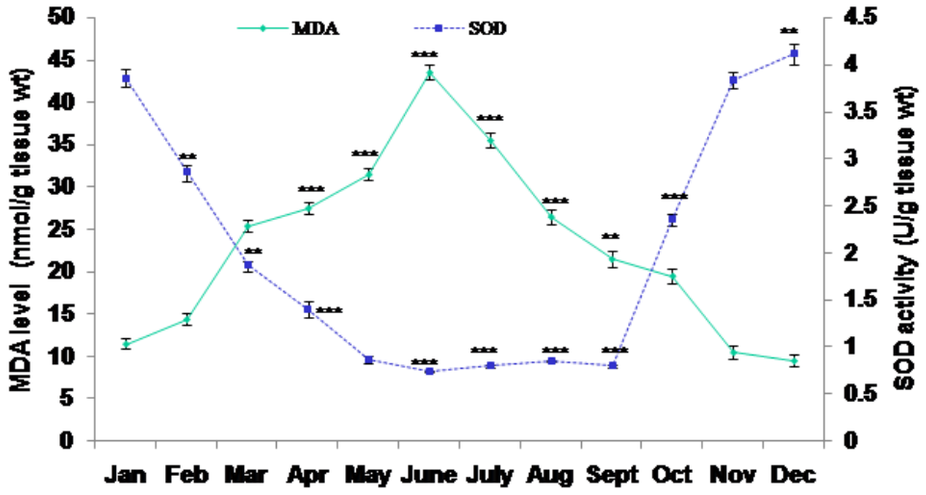

Fig. 3b. Annual variation in SOD activity and TBARS level in thyroid of *P. asiatica*.Data of each point represents mean±SEM; N=7. Vertical bar at each point represents standard error. *p<0.05, **p<0.01, ***p<0.001.The data for January were treated as control and compared with other months.

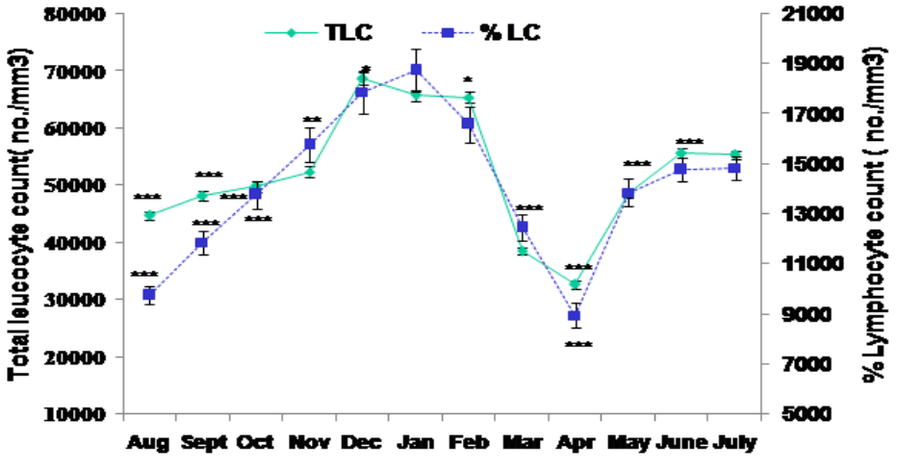

Fig. 4a. Annual variation of total leukocyte count (TLC) and percent lymphocyte count (% LC) of *P.asiatica*.Data at each point represents mean±SEM; N=7. Vertical bar at each point represents standard error. *p<0.05, **p<0.01, ***p<0.001.The data for January were treated as control and compared with other months.

Annual variation in total leukocyte count (TLC) and percent lymphocyte count (%LC)

There was a significant increase in TLC during August to February ($p<0.01$) when compared to January. The maximum total leukocyte count was observed in December (68789±789mm^3) and the minimum total leukocyte (32686±634mm^3 was observedin April. On the other hand a significant increase ($p<0.05$) in % LC was observedduring September to January. The maximum % LC was observed in January (18760±876mm^3 and minimum %LC (8958±489 no./mm^3) was observed in April (Fig.4a).

Annual variation in heterophil/lymphocyte (H/L) ratio

Variations in the H/L ratio were recorded through the year. There was a significant increase ($p<0.05$) in H/L ratio during the period May to August when compared to January. The maximum

H/L ratio was recorded in January (12±0.56) and the minimum H/L ratio (3±0.35) was recorded in March (Fig.4b).

Histomorphology of thyroid gland during the reproductively active phase (RAP) showed high thyroidal activity as judged from of the tall follicular epithelium and scanty colloidal substance in the lumen. On the other hand, during the reproductively inactive phase (RIP) the short follicular epithelium and the large amount of colloid in the lumen indicated hypo-active condition of the thyroid gland (Fig. 5A, B).

A parallelism between the thyroid and testicular histomorphology was observed. During RAP the testicular activity was high showing large seminiferous tubules, active spermatogenesis and abundant sperm in the lumen. Contrary to this, during RIP the seminiferous tubules were regressed, the spermatogenic activity was depleted, the spermatogonial cells were vacuolated and the lumen was filled with debris (Fig. 5C, D).

Discussion

P. asiatica is a long day breeder, a tropical avian species of northern India (Varanasi). The

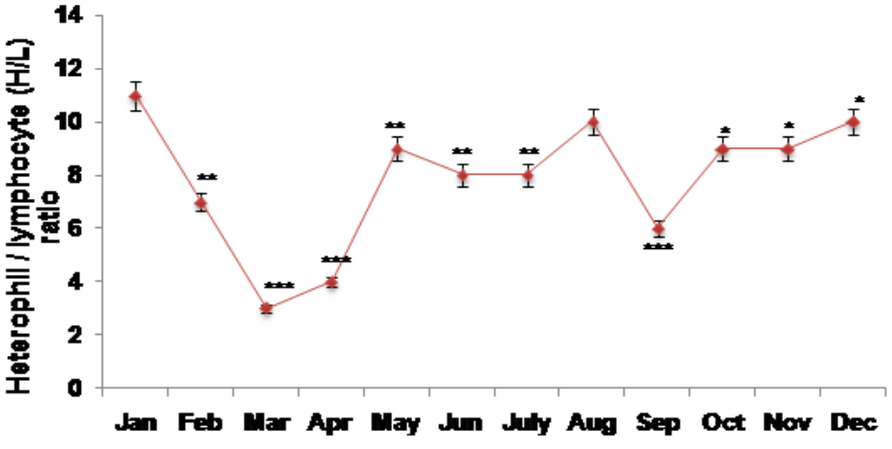

Fig. 4b. Annual variation of heterophil and lymphocyte ratio (H/L ratio) of splenocytes of *P.asiatica*. Data at each point represents mean±SEM; N=7. Vertical bar at each point represents standard error. *p<0.05, **p<0.01, ***p<0.001.The data for January were treated as control and compared with other months.

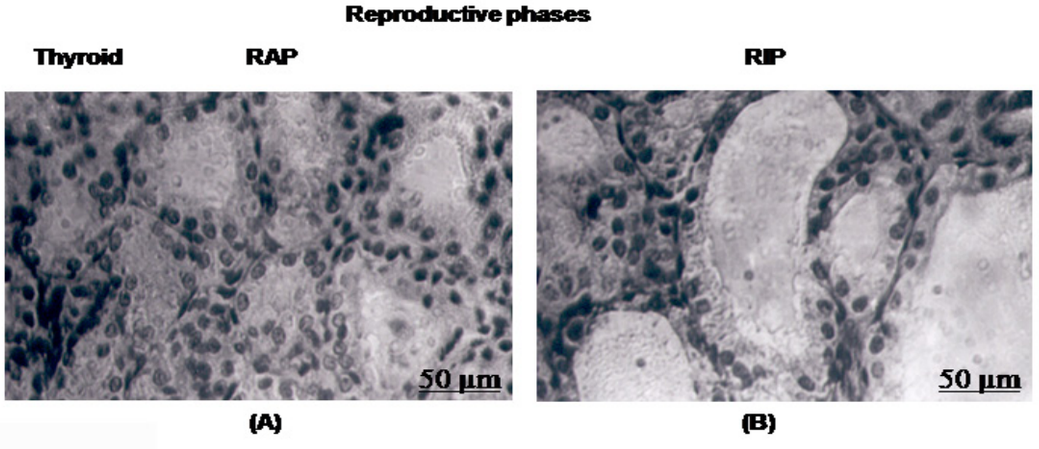

Fig. 5a, 5b. Histomorphology (H & E staining) of thyroid gland of P.asiatica showing active and inactive thyroid follicles during reproductively active phase { RAP; (A)} and reproductively inactive phase { RIP; (B)}, respectively. Scale bar = 50 µM.

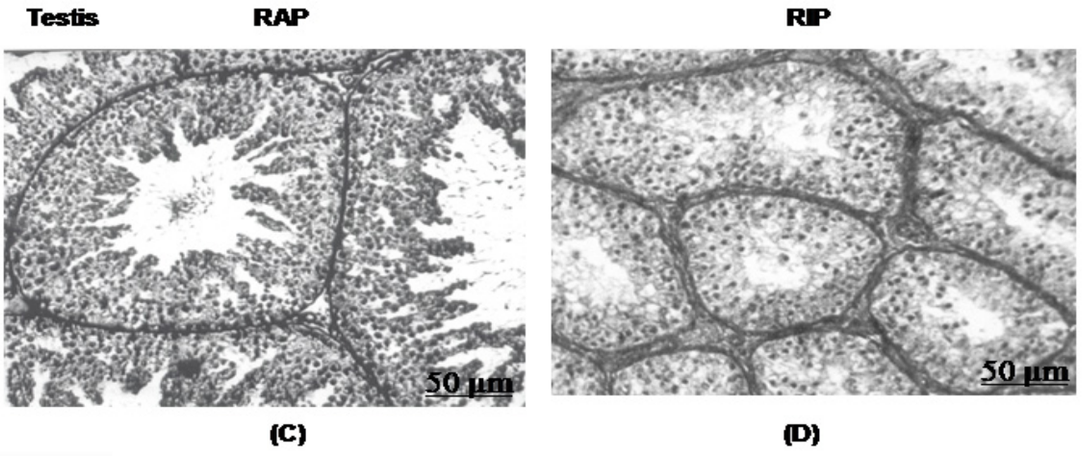

Fig. 5c, 5d. Histomorphology (H & E staining) of testis of P. asiatica showing active and inactive seminiferous tubules of testis during reproductively active phase { RAP; (C)} and reproductively inactive phase { RAP; (D)}, respectively. Scale bar = 50 µM

variation of the day length in this part of subtropical region is only 1 hr 45 min between summer and winter. However, other tropical environmental factors such as temperature (summer 48 °C, rainfall, winter 5 °C, and humidity in association, presents a drastic variation when compared with the temperate zone. A variation of approximately two hours between summer and winter in temperatures might have been perceived by the pineal gland of this bird to present the reproductively active period during summer and reproductively quiescent period during winter. Once the temperature increases from March onwards, a progressive phase of testicular activity sets in till May-June when peak testicular function has beenfound. The adult birds, in order to provide sufficient food for the young ones, migrate with the young ones to the plain regions where the grains are being harvested and also to overcome the harsh winter, a period of reproductively quiescent phase. It is well known that the pineal gland is involved in synchronizing various aspects of physiology related with changes in external environments (Krause and Dubocovich, 1990). Further, studies have established that pineal gland inhibits thyroid function (Haldar and Pandey, 1988; Haldar and Shavali, 1998).The gonadal active phase corresponds to lessened activity of the pineal when days are long and the nights are short whereas gonadal regressionphase corresponds to elevated activity of pineal gland, during which the nights are long and days are short. The variations in the levels of circulatory testosterone support the above explanation. The annual pattern in the secretion of gonadal steroid in relation to melatonin has been described in certain avian species (Haldar and Ghosh, 1990).

A parallel correlation of T3 and T4 levels with that of the thyroid gland weight was noted. During winter months when the birds are reproductively quiescent, with low metabolic rate, the thyroid activity was also low and *vice versa* during the sexually active period.In spite of drastic changes in the climate, the relationship between pineal and thyroid in tropical vertebrates appeared to be similar as in the temperate zone. Independent of the different vertebrate classes (mammal/birds/ reptiles), the pineal gland showed an inhibitory influence on the thyroid function (Haldar and Ghosh, 1990; Haldar and Srivastava, 1992; Haldar and Shavali, 1998). We also measured the H/L ratio or leukocyte count, a useful stress parameter, as it is altered by natural stressors (Dhabhar et al., 1996). Our results show that the H/L ratio is high during winter month (January and February) and low during March and April. Further, we observed a decrease in testicular weight, testosterone level, thyroid gland weight and plasma level of the thyroid hormones during the quiescent phase of the annual reproductive

cycle; however, the decrease was more prominent when the endogenous level of melatonin remained high in comparison to the active phase. The data on thymidine kinase activity indicates a decline in the level of H^3-Thymidine uptake by the thyroids during November to January, being the lowestin the month of November, the period corresponding to the sexually quiescent phase.This observation may be correlated with the inverse relationship between pineal and thyroid glands reported earlier (Haldar and Ghosh, 1990). Increasing trend was observed during February onwards till June, when the activity of the pineal gland was low and thyroid was not under the inhibition of pineal gland as suggested by Haldar and Ghosh (1990).The results suggest that the pineal gland exerts an inhibitory effect on the thyroid gland H^3-thymidine uptake during the quiescent phase of reproduction. The studies on annual variation in SOD activity suggested that from October to January the SOD activity was significantly high as compared to April to September. The decline and elevation in this enzyme activity revealindirect relationship with melatonin level as reported previously. Our results show that the higher melatonin level during October to February could be responsible forelevation in SOD activity during these periods.

Studies on annual variation in the malonaldehyde (MDA) level, an index of lipid peroxidation (LPO), indicates a steady decline during July to November, being the minimum during November and December. These results also suggest a direct protective role of melatonin against ROS during winter months when the photoperiod is short and the temperature is low and hence the level of melatonin is high (Haldar and Ghosh, 1990). The MDA level was high during March to August under long photoperiod and low peripheral melatonin concentration. During winter the peripheral melatonin is high in the bird and hence MDA level is reduced.

Based onthe above results we suggest that variation in peripheral melatonin due to change in natural light conditions strengthens the immune function on one hand and suppresses the gonadal activity in winter on the other to help the individuals to dealwith the seasonal stressors (food scarcity and low ambient temperature) and seasonal oxidative load. Hence, fluctuating immune function noticed throughout the year might be responsible for adaptations that have evolved to decrease the odds of survival and hormonal availability for reproduction.

Acknowledgments

The authors thank to the Department of Science and Technology (Order no-SR/SO/AS-36/2011), New Delhi, for financial assistance to Mr. V. K. Verma as project SRF, and the gift of instruments from Alexander von Humboldt Foundation, Bonn, Germany, to Prof. C. Haldar.

References

Csernus V, Mess B. (2003) Biorhythms and pineal gland. *Neuroendocrinol Lett*. **24**: 404-411.

Das K, Samanta L, Chainy GBN. (2000) A modified spectrophotometric assay ofsuperoxide dismutase using nitrite formation by superoxide radicals. *Indian J Biochem Biophys*. **37**: 201–204.

Dhabhar FS, Miller AH, McEwen BS, Spencer RL. (1996) Stress-induced changesin blood leukocyte distribution. Role of adrenal steroid hormones. *J Immunol*. **157**: 1638–1644.

Johansson AA, Eriksson L, Soveri T, Laakso ML. (2001) Seasonal variation in endogenous serum m elatonin profiles in goats: A difference between spring and fall? *J Biol Rhythms*. **16**: 254-263

Francis JP. (2014) On the value of seasonal mammals for identifying mechanisms underlying the control of food intake and body weight. *Horm Behav*. **66**: 56–65.

Garcia A, Landete-Castillejos T, Zaraga L, Garde J, Gallego L. (2003) Seasonal changes in melatonin concentrations in female Siberian red deer (*Cervus elaphus hispanicus*). *J Pineal Res*. **34**: 161-166.

Gupta BBP, Thapliyal JP. (1991) Endocrine regulation of the oxidative metabolism in poikilothermic vertebrates.*Zool Sci.* **8**: 625-634.

Gwinner E. (1989) Melatonin in the circadian system of birds: model of internal resonance. In: Hiroshige T, Honma K, eds. *Circadian Clocks and Ecology*. Sappora, Japan: Hokkaido Univ Press. pp. 127-145.

Haldar C, Ghosh M. (1990) Annual pineal and testicular cycle in the Indian jungle bush quail, *Perdicula asiatica*, with reference to the effect ofpinealectomy. *Gen Comp Endocrinol.* **2**: 73–81.

Haldar C, Pandey R. (1988) Effect of pinealectomy and photoperiod on testes and thyroid gland of Indian tropical palm squirrel, *F. pennanti*. *Indian J Expt Biol*. **26**: 516-519.

Haldar C, Rai A. (1997) Photoperiod, indole-amines, and ovarian responses inthe Indian tropical jungle bush quail, *Perdicula asiatica*. *J Exp Zool.* **277**: 442–449.

Haldar C, Shavali SS. (1998) Effects of continuous light, continuous darkness and pinealectomy on pineal-thyroid-gonadal axis of the female Indian palm squirrel, *Funambulus pennanti. J Neural Transm***. 105**: 407-413.

Haldar C, Srivastava M. (1992) Gonado-modulatory effects of high humidity is pineal dependent in Indian palm squirrel *Funambulus pennanti. Indian J Exp Biol.* **30**: 264-270.

Krause DN, Dubocovich ML. (1990) Regulatory sites in the melatonin system of mammals. *Trends Neurosci.* **13**: 464-470.

Morera AL, Abreu P. (2006) Seasonality of psychopathology and circannual melatonin rhythm. *J. Pineal Res*. **41**: 279–283.

Ohkawa H, Ohishi N, Yagi K. (1978) Reaction of linoleic acid hydroperoxide with thiobarbituric acid. *J Lipid Res*. **19**: 1053-105Ots

Murumagi A, Horak P. (1998) Haematological health state indices of reproducing great tits: Methodology and sources of natural variation. *Funct Ecol*. **12**: 700–707.

Ozturk G, Coskun S, Erbas D, Hasanoglu E. (2000) The effect of melatonin on liver superoxide dismutase activity, serum nitrate and thyroid hormone levels. *Jpn J Physiol*. **50**: 149-153.

Prakash P, Laloriya M, Kumar P. (1998) Influence of melatonin implant on the free radical load in avian thyroid and its relation with thyroid hormonogenesis. *Biochem Mol Biol Int*. **46**: 1249-1258.

Pevet P. (2000) Melatonin and biological rhythms. *Biol Signals Recept.* **9**: 203-212.

Reiter RJ, Maestroni GJ. (1999) Melatonin in relation to the antioxidative defense and immune systems: possible implications for cell and organ transplantation. *J Mol Med*. **77**: 36-39.

Rollag MD, Niswender GD. (1976) Radioimmunoassay of melatonin insheep exposed to different light regimes. *Endocrinology* **98**: 482–488.

Sudhakumari CC, Haldar C, Senthil Kumar B. (2001) Seasonal changes in adrenal and gonadal activity in the quail *P. asiatica*: Involvement of pineal gland. *Comp Biolchem Physiol. Part B. Biochem Mol Biol*. **1288**: 793-804.

Shinomiya A, ShimmuraT, Nishiwaki-Ohkawa T, Yoshimura T. (2014) Regulation of seasonal reproduction by hypothalamic activation of thyroid hormone. *Front Endocrinol.* **5**: 12.

Singh SS, Haldar C, Rai S. (2006) Melatonin and differential effect of L-thyroxine on immune system of Indian tropical bird *Perdicula asiatica*. *Gen Comp Endocrinol*. **145**: 215–221.

Todini L. (2007) Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors. *Animal*. **7**: 997–1008.

Vaughan GM, Pruitt BA. (1985) Pineal induced depression of free thyroxine in Syrian hamsters. *J Pineal Res*. **2**: 325-330.

Wajs E, Lewinski A. (1992) Inhibitory influence of late afternoon melatonin injections and the counter-inhibitory action of melatonin-containing pellets on thyroid growth processes in male Wistar rats: Comparison with effects of other indole substrates. *J Pineal Res*. **13**: 158-166.

Wood S, Loudon A. (2014) Clocks for all seasons: unwinding the roles and mechanisms of circadian and interval timers in the hypothalamus and pituitary. *J Endocrinol.* **222**: 39–59.