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Changes in food intake, body weight, gonads and plasma concentrations of thyroxine, luteinizing hormone and testosterone in captive male buntings exposed to natural daylengths at 29° N

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Abstract. We have investigated the seasonal changes in food intake, body weight, gonadal volume and plasma concentrations of thyroxine, luteinizing hormone and testosterone in male blackheaded bunting (*Emberiza melanocephala*) in captivity under natural daylengths at 29° N. The cycles in food intake, body weight and testis size in buntings appeared to be phase related. While the changes in body weight and testicular size were parallel to each other and correspond to the increasing daylengths of spring and early summer, cycle in food intake was almost antiphase to the cycles in body weight and testicular growth and development. Furthermore, buntings showed a distinct seasonal cycle in plasma concentrations of thyroxine, luteinizing hormone and testosterone. It is suggested that these seasonal cycles in buntings are endogenously programmed and their entrainment to the environmental photoperiod ensures the occurrence of different physiological functions at temporally fixed time of the year.

Keywords. Body weight; food intake; testis; thyroxine; luteinizing hormone; testosterone; *Emberiza melanocephala.*

1. Introduction

The regulation of annual periodicity in physiological and behavioural processes in wild birds depends upon the appropriate phase relationship among several endogenous components, including secretion of various hormones from the endocrine glands. Many, if not all, endogenous components need entrainment with the environmental variable(s), which is(are) precise in occurrence (in many cases, environmental variable is the daylength or photoperiod), to keep them in proper pace with seasons of the year. Less is known of the seasonal periodicity in physiology and endocrinology of birds which overwinter in India (but see Pathak and Chandola 1982).

The present study was done on the blackheaded buntings (*Emberiza melanocephala*) which arrive in India during fall (September/October), overwinter, and return to their breeding grounds in western Asia and eastern Europe (about 40° N) during late spring (March/April) (Ali and Ripley 1974). This paper reports the seasonal changes in food intake, body weight, gonadal volume and plasma concentrations of metabolic and reproductive hormones in the male blackheaded buntings maintained in captivity under natural daylengths (NDL) at 29° N. The results further our understanding of the physiology of the seasonal events in birds, particularly in

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migratory species that overwinter at low latitudes, since no such comprehensive study has been performed on any palaearctic–Indian migratory form.

2. Materials and methods

2.1 *Birds and their maintenance*

Adult male bunting caught from the overwintering flocks at Varanasi (25° N) during early winter were brought to Meerut (29° N) and placed in an outdoor aviary. Later, they were caged (n = 6 per cage; size = 45 × 25 × 25 cm) and kept in a room that received natural light through large windows and glass panes, allowing them to acclimate to the laboratory conditions for two weeks before beginning of the experiment.

In all, six groups of acclimated birds were used in the present study which was completed in two years. The study included observations on the changes in food intake, body weight, testis size and endocrine secretions [namely, plasma concentrations of thyroxine (T_4), luteinizing hormone (LH) and testosterone (T)] in relation to the annual changes in daylength at 29° N. The seasonal variations in daylength at 29° N (Meerut) occur between the shortest daylength of 11 h 05 min (22nd December) and the longest daylength of 14 h 57 min (21st June); this includes 24–28 min of morning and evening civil twilight period (figure 1) (Nautical Almanac, National Astronomical Laboratory at Calcutta).

2.2 *Food intake and body weight*

Data on the changes in food intake (FI) and body weight (BW) were obtained in two years, first in between March and August 1991 and then again in between January and September 1992. The data from both the years were pooled together for further analysis.

FI was measured in birds (n = 6) housed singly in cages. The cages lined by opaque white polythene sheets on sides up to 5 cm above the perch level and placed in steel trays which facilitated spillage collections, were kept 20 cm apart from each other on a cemented shelf situated at a 1·0 meter height in the NDL room. A fixed amount of food (30 g per cage) was dispensed in food cups every fourth day. The difference between the initial and final weight of food cups gave the amount of food consumed by a bird in 72 h period. From this, FI over 24 h period could be calculated for each bird and then meant ± SEM for the group was calculated. Changes in body mass was also recorded every fourth day by weighing an individual on a top balance providing accuracy to the nearest 0·1 g.

2.3 *Testicular size*

The dimensions of left testis in a group of 10 birds as inspected by laparotomy under local anesthesia at monthly intervals during the period between January and

Figure 1. Changes (mean ± SEM) in/food intake **(A),** body weight **(B)** and testicular volume **(C)** of blackheaded buntings in relation to changes in daylength at 29° N (shown in the top panel). Observations were made biweekly on food intake and body weight, and monthly on testicular volume from late January to early September. Mean and Standard error of the data for a group of birds are represented by a circle and a vertical bar, respectively.

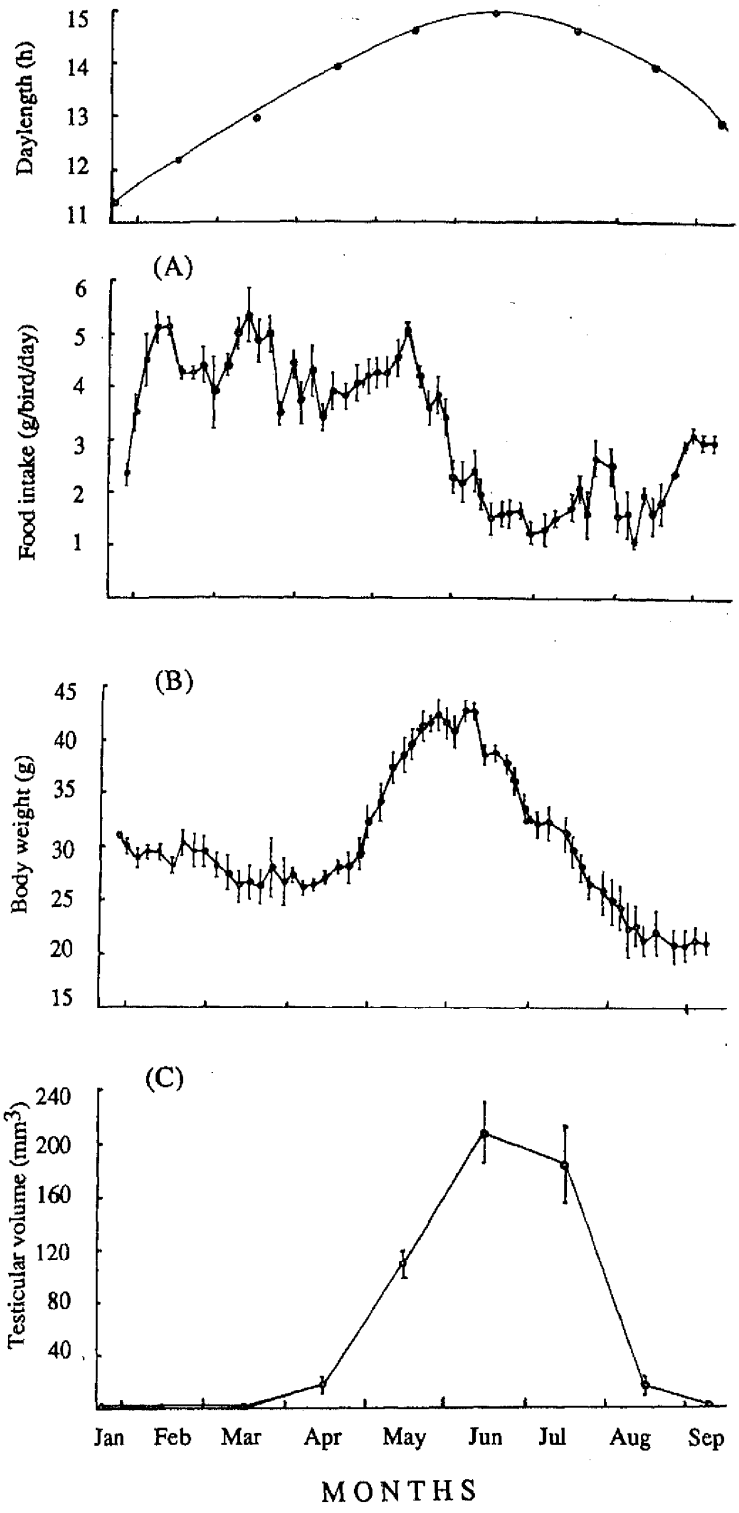
Daylength (h)
15
14
13
12
11
(A)
Food intake (g/bird/day)
6
5
4
3
2
1
(B)
Body weight (g)
45
40
35
30
25
20
15
(C)
Testicular volume (mm3)
240
200
160
120
80
40
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
MONTHS

Figure.1

September 1992 were recorded. Testis volume was calculated using formula 4/3 πab^2, where a and b denote half of the long and short axes, respectively.

2.4 *Blood sampling and measurement of hormones*

At each time a sample of whole blood (100–150 µl) was collected from the wing vein into heparinized capillary tubes. All samples were centrifuged and the plasma supernatant were stored at –20°C until assayed. The measurement of hormones was done using specific radioimmunoassay (RIA) techniques, as described below.

2.4a *Measurement of plasma T_4:* Plasma concentrations of T_4 were measured at monthly intervals, between January and July, as per established method described in detail by Boswell (1991) for quail plasma and validated later for bunting plasma (Jain 1993). This three-day assay used an antiserum raised in sheep against thyroxine (S 196) which was obtained from RAST Allergy Unit, Benenden Chest Hospital, Cranbrook, Kent, UK. T_4 (T-2501) standard was obtained from Sigma Chemical Co., USA, and dissolved first into 0·1 M NaOH and then diluted down to the required concentrations in assay buffer (0·075 M barbital buffer at pH 8·6) for the purpose of the T_4 assay. Radioactive labelled thyroxine (code IM. 141, 250 µci) was obtained from Amersham International plc, Buckinghamshire, UK. A T_4 standard curve was prepared in 20 µl triplicates with ten dilutions of a top dose of 100 ng/ml (64·3 nmol/1), total binding and non-specific binding controls. For unknown samples, 20 µl of plasma aliquots in duplicate were used. A 20 µl of chicken ligand free plasma (LFP; we found no difference between LFP from chicken, quail and bunting plasma) was added to standard curve and a 20 µl of assay buffer to unknown samples. Antiserum (20 µl) at a concentration of 1 : 6000 in 1 : 400 normal sheep serum (NSS) in assay buffer was then added, followed by 20 µl of labelled hormone which was diluted in 1 mg/ml solution of 8-anilino-l-napthalenesulphonic acid (Sigma, USA, A-3125) in assay buffer. This mixture was incubated for 24 h at 4°C. On day 2, 20 µl of anti-sheep precipitating serum at a concentration of 1 : 30 in assay buffer was added to separate bound and free labelled hormone and the mixture was incubated again at 4°C for another 24 h. On the final day (day 3), 200 µl of assay buffer was added to each tube, making the total volume of reaction mixture of 300 µl, and then centrifuged at 3500 *g* at 4°C for 45 min. The supernatant was aspirated and the precipitate counted in a gamma counter. Plasma concentrations of T_4 were then calculated with reference to the Standard curve using a microcomputer program for T_4 assay written in Laboratory at Bristol, UK, and run on a BBC basic computer. There was close parallelism between a thyroxine Standard curve and a bunting's plasma dilution curve. Acceptable range of this assay was in between 90% and 10% binding levels (in between 0·25 ng/ml and 25 ng/ml). Thus, the lower limit of detection of the assay (i.e., the sensitivity of the assay, 90% B/BO) was 0·25 ng/ml (5 pg/tube). Inter- and intra-assay variation was 13·2% and 6·9% respectively ($n = 8$).

2.4b *Measurement of plasma LH*: Plasma concentrations of LH were measured at 2-week intervals in between January and October by micromodification of the method developed by Follett *et al* (1972) and validated for bunting's plasma (Jain 1993; Kumar *et al* 1993), using duplicate plasma samples (20 µl/sample) and original

chicken LH fraction IRC-2 as standard. There was close parallelism between a chicken LH standard curve and a bunting's plasma dilution curve. The sensitivity (i.e., 90% B/BO) of the assay was 0·l0 ng/ml (2 pg/tube). Inter- and intra-assay variation was 13·5% (n = 6) and 8·8% (n = 8) respectively.

2.4c *Measurement of plasma T*: Plasma concentration of T were measured at 2-week intervals in between January and October, using method of Boswell (1991) and later validated for bunting's plasma (Jain 1993) (for details please refer to Boswell *et al* 1995). However, briefly, this assay used a highly specific commercial antiserum (cross reactivity: 5α-dihydrotesiosterone, 0·54%; 5α-androstan-3ß ol-17-one, 0·007%; estradiol- 17β, 1·2*%;* estriol, 0·01%; estrone, 0·07%: progesterone, 0·004%; corticosteroid, 0·0016%; deoxycorticosterone, 0·007%) raised in sheep (P. 15·3·82) which was obtained from RAST Allergy Unit, Benendan Chest Hospital, Cranbrook, Kent, UK. Tritium-labelled testosterone was obtained from Amersham. Concentrations of T were measured in duplicate plasma samples (50 µl/sample) which were extracted with diethyl ether (efficiency 85–98%), dried under nitrogen, and reconstituted in phosphate-buffered saline (PBS) in duplicates (50 µl each). The Standard curve (from testosterone, Sigma Chemical Co. USA, dissolved in ethanol and diluted in 0·05 M PBS), the total binding and non-specific binding controls were treated in an identical manner to the sample tubes. Reconstituted extracts were incubated with 50 µl each of antiserum (1:10,000 in PBS) and labelled testosterone, and 50 µl of anti-sheep precipitating serum (1 : 15 in PBS) was used to separate bound and free labelled hormone. There was close parallelism between a testosterone standard curve and a bunting's plasma dilution curve. The lower limit of detectabilily of the assay (i.e., the sensitivity of the assay, 90% B/BO) was 0·06 ng/ml (0·12 pg/tube). Inter- and intra-assay variation was 15·4% and 9·2% respectively (n = 8).

2.5 *Statistics*

Data were analysed using analysis of variance (ANOVA) with repeated measures followed by Post-hoc Duncan's Multiple Range Test (DMRT) for comparison within the group, if ANOVA indicated a significant difference. The data on FI and BW were divided into 4-week (8 observations) blocks and then statistical analysis was done using block means.

3. Results

3.1 *Food intake and body weight*

Biweekly changes in FI over an 8-month period, beginning from late January, are shown in figure 1 A. FI was low (mean ± SEM FI bird^{-1} day^{-1} = 2·35±0·23 g) at the beginning, but then increased consistently reaching to the maximum (mean ± SEM FI bird^{-1} day^{-1} =5·15 ± 0·15 g) by mid-February, It remained high until mid-May and subsequently declined gradually over the period between mid-May and early September reaching as low as (mean ± SEM bird^{-1} day^{-1}) 1·09±0· 1 1g, except a small surge in FI during late July and during late August/early September. ANOVA

of the data also indicated a significant effect of time on FI ($P < 0.01$, F value = 7·20 at df 6, 60). A comparison of block means revealed a significant difference (DMRT $P<0·05$, $P < 0·01$ -January to May vs June to August) in food intake over different seasons.

Bunting had normal weight (24–28 g) until April and then showed a gradual increase reaching to maximum weight (up to 53·0 g) during May and early June. They lost weight ($P < 0·01$) by late June and gradual decrease in body weight continued until August when birds weighed as low as 16–17 g (figure 1B). ANOVA of the data indicated that the changes in body weight over the months of study were highly significant ($P = < 0·01$, F value = 12·80 at df 6, 60). A comparison of block means supported the significance in difference ($P < 0·05$, $P < 0·01$; DMRT) observed in changes in body weight over the period between January and September.

3.2 *Testis size*

Testes were small (testicular volume, TV = 0·35–0·57 mm^3) up to March and then began to enlarge (TV = 17·11 ± 6·07 min^3) in April (figure 1C). Testes grew in size and reached to peak size in June (TV = 207·40± 23·97 mm^3). Testicular regression began in July and significantly regressed ($P < 0·01$) testes (TV = 2·80±1·40 mm^3) were found in August (daylength = 14:00h). All birds had completely involuted testes (TV = 0·35 mm^3) by September. ANOVA of the data indicated significant effect ($P < 0·002$, F value = 29·26 at df 7, 21) of the time on testicular size in bunting. Comparison of mean TV between different months revealed significant differences ($P < 0·01$, DMRT).

3.3 *Thyroxine*

Plasma thyroxine (T_4) levels (0·64 ± 0·18 ng/ml) found in January remained significantly unchanged till April. A significantly increased ($P < 0·05$, DMRT) T_4 levels were found in May and subsequently plasma T_4 levels peaked in June (5·05 ± 1·3 ng/ml), a nearly 6-8-fold increase in plasma T_4, followed by decrease in plasma T_4 levels in July (figure 2A). ANOVA of the data indicated significant variation ($P < 0·05$, F value = 8·88 at df 6, 42) in plasma T_4 concentrations over the period of study. Pairwise comparison of means indicated significant difference ($P < 0·01$) in plasma T_4 levels between June/July and other months of the study.

3.4 LH and T

Plasma concentrations of LH were low (0·10 ± 0·02 ng/ml) in January, rose consistently in February through June and peaked in June (plasma LH = 1·09± 0·21 ng/ml) (figure 2B).

Figure 2. Changes (mean ± SEM) in plasma concentrations of thyroxine, T_4 **(A),** luteinizing hormone (LH) **(B)** and testosterone (T) **(C)** in blackheaded buntings in relation to changes in daylength at 29° N (cf. curve on daylength of figure 1) . Data on plasma concentrations of T_4 on a group (n = 4–8) of birds held under NDL were collected at monthly intervals from late January to late July. Observations on plasma concentrations of LH and T on a second group of birds (n=10) held under NDL were made from blood sample collected at 2-weekly intervals from mid-January to mid-October. Mean and standard error of the data for a group of birds are represented by a circle and vertical bar, respectively.

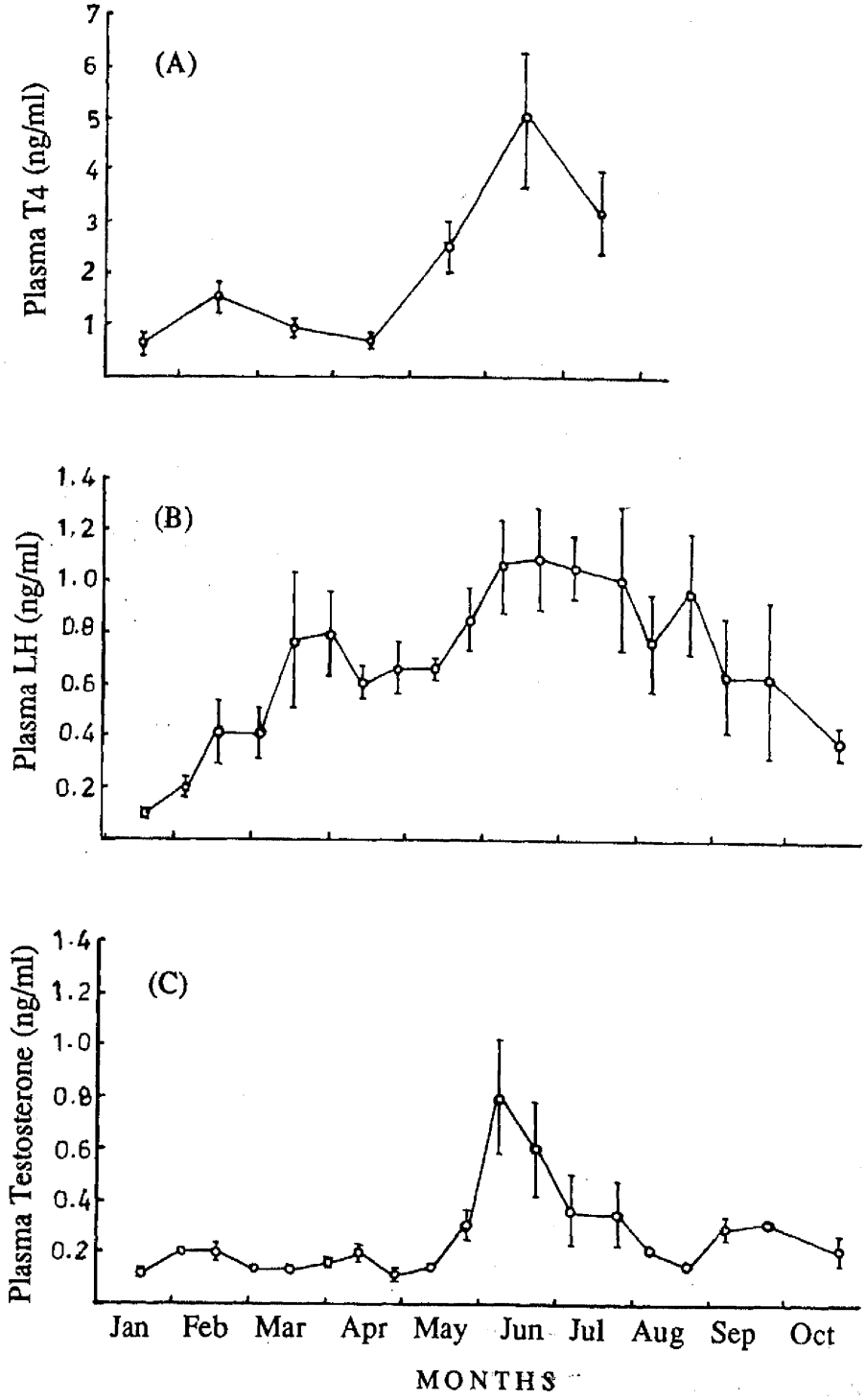
(A)
Plasma T4 (ng/ml)
7
6
5
4
3
2
1
(B)
Plasma LH (ng/ml)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
(C)
Plasma Testosterone (ng/ml)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Jan Feb Mar Apr May Jun Jul Aug Sep Oct
MONTHS

Figure 2.

It began to decline gradually and attained low levels (0·39± 0·06 ng/ml) in October. ANOVA of the data indicated that the changes in plasma concentrations of LH were significant over the period between January and October *(*$P < 0{\cdot}01$, F value =3·91 at df 18, 162). Plasma LH concentrations in birds during June/July were significantly higher than that during January, February, March and October *(*$P < 0{\cdot}05$, $P < 0{\cdot}01$; DMRT). Choosing an arbitrary plasma LH value of 0·5 ng/ml as base line plasma LH level, LH values below and above which were considered as low and high, respectively, plasma LH peak could be defined: the plasma peak was broader in shape and occurred in between June and July (figure 2B).

Plasma concentrations of T were low (0·12± 0·02 to 0·16 ± 0·02 ng/ml) from January to mid-May and then attained peak levels (0·79 ± 0·22 ng/ml; n=7) in June (figure 2C) . Plasma T levels declined and reached to minimum (0·15 ± 0·01 ng/ml; n = 3) in August. There was a significant effect ($P < 0{\cdot}005$; F value = 3·14 , at df 18, 108) of time (of exposure to NDL) on plasma T concentrations, as indicated by ANOVA. Comparison of means indicated that the plasma testosterone levels in birds in June were significantly higher ($P < 0{\cdot}05$) as compared to those found during the other periods of study.

4. Discussion

Results from the present study should be viewed against the background that the seasonal changes in physiological and behavioural responses in organisms are the resultants of the interactions between the endogenous and exogenous factors. The data (figures 1, 2) indicate a distinct seasonality in food intake, body weight, testis growth and development, and endocrine secretions (LH, T and T_4) in buntings, relative to the annual variations in daylength at 29° N. While the cycles in body weight and testicular size run parallel to each other and correspond to the increasing daylengths of spring and early summer, the cycle in food intake is almost antiphase to the cycles in body weight and testicular growth and development (cf. figure 1A-C). A similar situation has been reported in the non-migratory spotted munia (*Lonchura punctulata*) (Bhatt and Chandola 1985). Some other non-migratory and migratory species of birds also exhibit similar responses (Gwinner 1981; Nair *et al* 1994),

We do not know the reasons precisely for an inverse relationship between the food intake and reproduction in the blackheaded bunting (cf. figure 1A, C). However, it could be due to (i) negative feedback effects of gonadal steroids on food intake in view of the reports in the redheaded bunting (*Emberiza bruniceps*) that testosterone propionate injections cause significant decrease ($P < 0{\cdot}05$) in food intake (Kumar and Kumar 1990), (ii) drastic changes in temperature during winter, spring and summer months, and/or (iii) some adaptive reasons as reproduction leaves little time for foraging etc., and low food intake during this period could be a part of broader strategy for survival of species. A role of steroids has also been documented in a few other birds (Peterson *et al* 1973; Donham 1979).

As the fat deposition and subsequent weight gain is accompanied with the active hyperphagia and gonadal recrudescence (cf. figure 1A–C), it could be reasoned that the periods of hyperphagia could be contributing to the fat deposition in buntings, as is true of many other migratory species (Wingfield and Farner 1980).

Changes in plasma concentrations of T_4 during January to April (figure 2A) are consistent with the seasonal plasma T_4 profiles of redheaded buntings (Pathak and Chandola 1982). It is also reported that low plasma T_4 levels are concomitant with high plasma T_3 levels (John and George 1978; Pathak and Chandola 1982). The net result of T_4 being low and T_3 being high is due to increase in T_3 /T_4 ratio which in turn determines the premigratory fattening (Pathak and Chandola 1982; Kar and Chandola 1985). In the blackheaded buntings, plasma T_3 concentrations are not known but based on its allied species, the redheaded buntings (*Emberiza bruniceps*) (Pathak and Chandola 1982; Kar and Chandola 1985), it is speculated that low plasma concentrations during the premigratory and prebreeding period indicate for a high T_3/T_4 ratio.

The results showing seasonal cycles in plasma concentrations of LH and T are similar to those reported in white-crowned sparrows (W[i]ngfield and Farner 1978), starlings (Dawson and Goldsmith 1982) and rooks (Lincoln *et al* 1980). That the increasing natural photoperiods induce rise in LH and cause gonadal growth in buntings has been confirmed under laboratory conditions (Kumar *et al* 1993). Importantly, the plasma LH also rose during the period of year when photoperiodic response system recovers from the period of photorefractoriness and testes still remain unstimulated.

In summary the present results show a distinct seasonal cycle in food intake, body weight, gonads and endocrine secretions in buntings, which may be interlinked or phase related. The entrainment of these cycles to the environmental photoperiod ensures occurrence of different physiological activities at temporally fixed time of the year.

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