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SEASONAL CHANGES IN THYROID ACTIVITY IN THE FEMALE SHEATH-TAILED BAT, *TAPHOZOUS LONGIMANUS* (CHIROPTERA: EMBALLONURIDAE)

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The present study was designed to investigate changes in thyroid activity during the reproductive cycle in *Taphozous longimanus*. Thyroid gland showed marked seasonal variation in weight and secretory activity. It was inactive in quiescence and early to mid-winter dormancy and active during recrudescence and breeding period during late winter dormancy. The serum 3,5,3'-triiodothyronine (T_3) and thyroxine (T_4) concentrations showed significant variation and closely coincided with thyroid activity. The T_3 and T_4 concentrations were higher in recrudescence, late winter dormancy and minimum in quiescence and initial stages of first pregnancy. The body weight ($r = 0.56$), ovary weight ($r = 0.73$), and thyroid weight ($r = 0.70$) showed correlation with each other and with T_3 and T_4 concentrations. The correlation between body weight, thyroid weight and T_3 and T_4 concentrations in non-pregnant bats was higher when compared with pregnant bats. The T_3 and T_4 levels remained low during the initial stages of development in first pregnancy when compared with the initial stages of second pregnancy. The scant food supply and low levels of T_3 and T_4 and low temperature during initial stages of first pregnancy might be responsible for differential rate of fetal development in two successive pregnancies in *T. longimanus*.

Keywords: Bats – Emballonuridae – *Taphozous longimanus* – thyroid – T_3, T_4, seasonal changes – reproductive cycle

INTRODUCTION

The Chiroptera, the second largest order of mammals, exhibit a variety of reproductive delays in their reproductive cycle, which have been reviewed from time to time [10, 13, 26, 27]. However, the detailed reproductive pattern and endocrine characteristics have been documented in only a few species, e.g. *Miniopterus schreibersii* [26], *Myotis lucifugus lucifugus* [2], *Miniopterus schreibersii fuliginosus* [22] and *Scotophilus heathi* [31]. In the family Emballonuridae, reproductive patterns have been described in some detail only in genus *Taphozous* (*Taphozous georgianus* [18] in Australia and *T. melanopogon* [16, 17], *Taphozous longimanus* [11, 19, 32] in India). The thyroid gland plays a crucial role in the growth, development, and metabolism of nearly all the tissues [4]. The embryonic growth and the pregnancy has been

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reported to be influenced by maternal thyroid hormone [22, 25]. It has been also suggested that the level of plasma gonadotrophin was associated with hypothyroidism [6, 12]. Instead of many unique features, studies related to thyroid function in bats are limited and the few available studies, are restricted to the temperate region bats [7, 14, 15, 20, 34, 35]. *T. longimanus* is a subtropical emballonurid bat; it shows differential rate of fetal growth in its two successive pregnancies [19].

In a recent study [19] this species presents delayed development of the fetus during first pregnancy. Pregnant females were observed from mid-January to mid-August. First pregnancy lasted 119 ± 5 days and the successive second pregnancy lasted 90 ± 5 days. Varanasi, India displays subtropical conditions; the cold season lasts from November to February; extremely cold season lasts during mid-December to January (mean low, 4° ± 2 °C, mean high, 16° ± 4 °C). During this period insect population was lower then during other months of the year. Bats enter in winter dormancy during this period. The body weight increases due to accumulation of fat before entering into winter dormancy; this is similar to increase in body weight before hibernation in temperate-zone bats. During this period bats exhibit hypothermia (with an anal temperature lower than at other period of the year) and stop foraging. The delay in fetal development in first pregnancy coincides with low food availability, low temperature and increased body metabolism. To our knowledge no data related to thyroid activity in reproduction are available on any emballonurid species. Therefore, the aim of the present study was to investigate the changes in thyroid gland activity and T_3/T_4 concentration during the reproductive cycle of the female sheath-tailed bat, *T. longimanus*.

MATERIALS AND METHODS

Collection of animals

All experiments were conducted in accordance with the principals and procedures approved by the departmental research committee at Banaras Hindu University, Varanasi, India. Bats were available throughout the year in Varanasi, India (25° N, 83 °E). Adult female *Taphozous longimanus* were trapped alive at the University campus and adjacent areas. Twelve bats were captured in the first week of every month between 6 to 8 AM beginning from July 1997 until June 1999. Twelve bats were used for T_3 and T_4 assays (two animals pooled for one parameter) and six animals were used for histology of the thyroid. Bats with less than 20 gm body weight, wing span less than 40 cm, undeveloped gular gland and darker pelage coloration were considered immature and were not used. The body weight of the adult bats was recorded within 2 hours of capture. Bats were anesthetized with diethyl ether before they were decapitated and their blood was collected. Since these bats are small the blood from two bats was pooled to get 1 ml of blood. The blood stayed for 1 hour at room temperature for clotting. It was then centrifuged at 1800 × g for 20 minutes. Serum was separated and stored at –20 °C until it was assayed.

Histology

Ovaries (n = 6), and thyroid (n = 6) were dissected out, freed from excess fat, weighed and fixed in Bouin's solution for 48 h, stored in 70% alcohol, dehydrated in ethanol, cleared in xylol, and embedded in paraffin; the tissues were serially sectioned at 6 μm and stained with hematoxylin and eosin. Based on the reproductive cycle of *T. longimanus* [19] and the ovarian microstructure, females were classified in the following five categories: (1) Quiescence (August–September): pre-antral follicles; (2) Recrudescence (October–November): antral follicles; (3) Winter Dormancy (December–early January): both pre-antral and antral atretic follicles; large number of polyovular follicles; (4) first pregnancy (mid-January to mid-May): large antral follicles; ovulation and mating takes place during this period. Embryos showed retarded growth and (5) second pregnancy (mid-May–mid-August): healthy and atretic follicles were noticed during this period. Embryos showed rapid growth during second pregnancy. The presence of functional corpus luteum confirmed the pregnant status.

T_3 and T_4 assays

Circulating concentrations of T_3 and T_4 were measured by radioimmunoassay (RIA) using RIAK 5 human kit obtained from Bhabha Atomic Research Center, Bombay, India. It has been our experience that chiropteran peptide hormone shows greatest cross-reactivity with antibodies generated against human. This has been reported and validated from our laboratory for T_3 and T_4 measurements in another species, *S. heathi* [20]. The T_3 and T_4 assays were performed by adding 100 μl serum samples for T_4, and 50 μl serum samples for T_3 to 100 μl of labelled thyroid hormone (approximately 10,000 counts per minute), mixing them and then adding 100 μl of the respective antiserum. The mixture was stirred and incubated for 2 h at room temperature for T_4 and 3 h for T_3 as described by the manufacturer's protocol. After incubation, the bound antigen for T_3 and T_4 were separated by adding 1 ml of polyethylene glycol. The precipitate was collected and radioactivity determined by Gamma counter. The concentration range of standard curves for T_3 covered 0–400 ng and for T_4 0–2000 ng. The intra-assay coefficients of variation were 7.4% for T_3 and 8.6% for T_4 and inter-assay coefficient of variation were 10.7% for T_3 and 11.3% for T_4. The cross-reactivity of T_4 to T_3 antiserum was 2% to other major steroids, while that of T_3 to T_4 was 1%. The cross-reactivity of these antisera to other related compounds was less then 0.1%.

Statistical analysis

Data were analysed by one-way ANOVA. If the analysis was significant then a Duncan multiple range test was run. When appropriate, correlation coefficient was applied. Differences were considered significant at $P < 0.05$. Data were presented as the mean ± SE.

RESULTS

Changes in body, ovary and thyroid weights

The body weight, ovary weight and thyroid weight in relation to pregnant status showed remarkable fat deposition as shown in Fig. 1. The body weight begins to increase in September and attains a peak in recrudescence due to accumulation of fat. The body weight declines in early to mid-winter dormancy and shows two peaks, one in April and the other in July (Fig. 1), respectively, at the time of advanced stage of two successive pregnancies. The ovarian and thyroid weight show two peaks during recrudescence, late winter dormancy. Thyroid weights began to increase from quiescence, attained a peak in recrudescence, and declined during early to mid-winter dormancy; increasing again in late winter dormancy and thereafter no significant change in the weights of both ovary and thyroid were observed during other months of the year. The thyroid weight declined during early embryonic development of first pregnancy. The thyroid weight declined during late stages of second pregnancy.

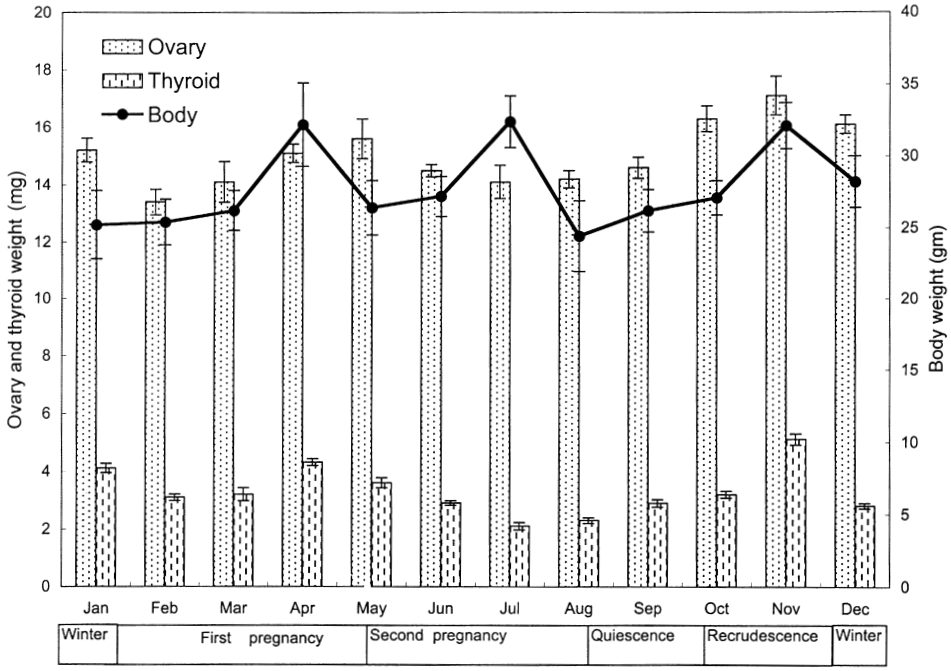

Fig. 1. Seasonal changes in body, ovary and thyroid weight during reproductive cycle of female bat, *T. longimanus;* n = 6 for each parameter. Values are mean ± SE. Values are significantly different using Duncan's test. Body weight: November, April and July Vs. other months; ovary weight: November Vs. other months of the year; January vs other month of the year except November and May; thyroid weight: November Vs. others month of the year; January vs other month of the year except April

T_3 and T_4 concentrations

Serum T_3 and T_4 concentrations showed significant ($P < 0.05$) variation with changes in reproductive cycle (Fig. 2). Serum T_3 and T_4 concentrations began to increase from quiescence to a short peak in recrudescence. The concentration of both T_3 and T_4 declined during early to mid-winter as compared to recrudescence. A peak of T_3 and T_4 was noticed during breeding in late winter in January at the time of mating and implantation. T_3 and T_4 concentrations declined during the period of delayed embryonic development (February–March) in the first pregnancy as compared to initial stages of second pregnancy (May). A smaller peak of both T_3 and T_4 was noticed during initial stages of second pregnancy in May and thereafter a steady decline was

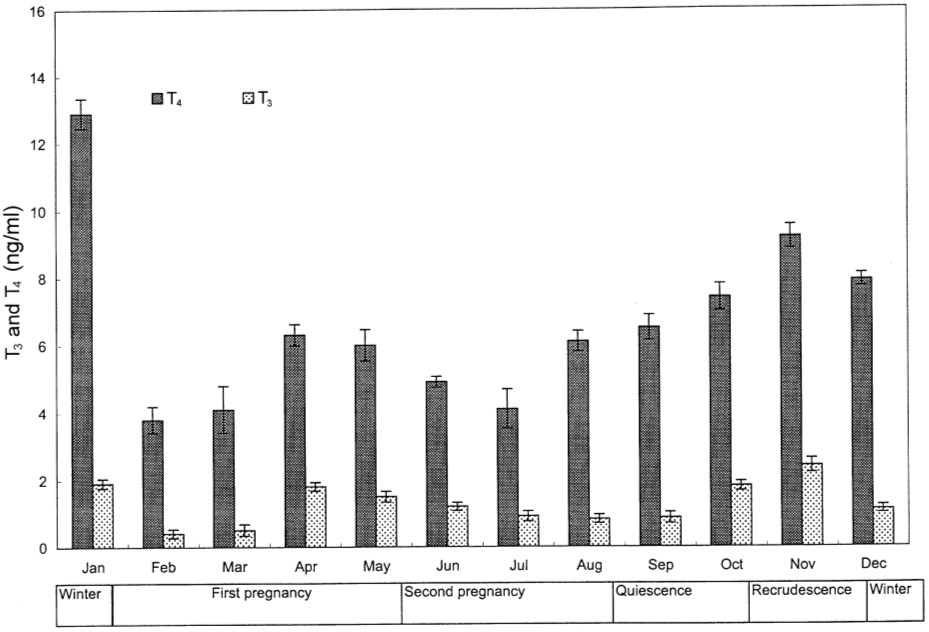

Fig. 2. Seasonal changes in T_3 and T_4 concentrations in female *T. longimanus*. Values are given as the mean ± SE. Values differ significantly ($P < 0.05$) as follows; T_4: January vs. other months; November vs. December; T_3: January vs. Other months; November vs. other months except April

noticed both in T_3 and T_4 till the late stage of second pregnancy. The correlation coefficient between circulating T_3 and T_4 concentrations with body, ovary and thyroid weights are shown in Table 1. T_3 and T_4 concentrations showed correlation with body, ovary and thyroid weights before early to mid winter in December ($r = 0.56$; $r = 0.76$; $r = 0.70$, respectively; Figs 3 and 4), while no correlation was observed between T_3 and T_4 with body, ovary and thyroid weights during the two successive pregnancies ($r = 0.15$; $r = 0.21$; $r = 0.23$), respectively.

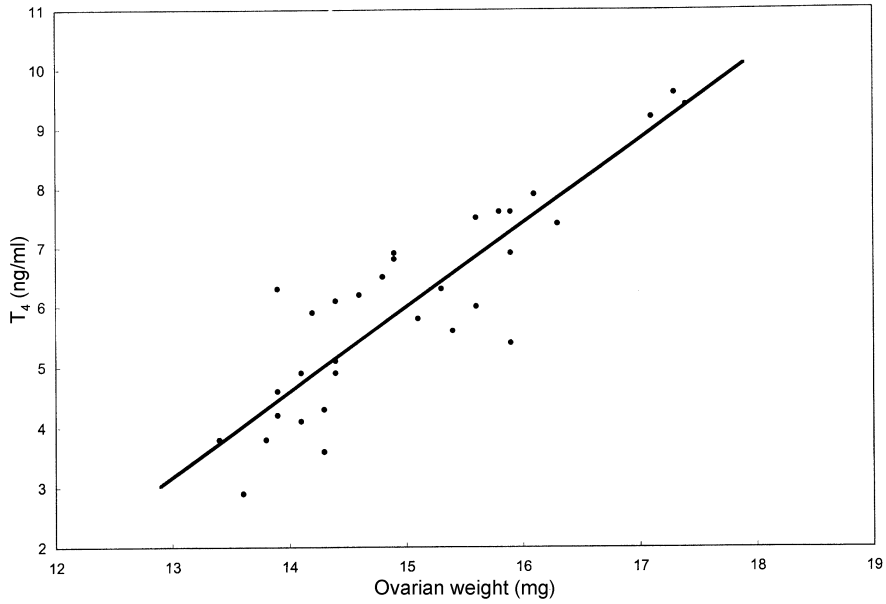

Fig. 3. Correlation between circulating T_4 concentration (ng/ml) with ovarian weight (mg) in female *T. longimanus*

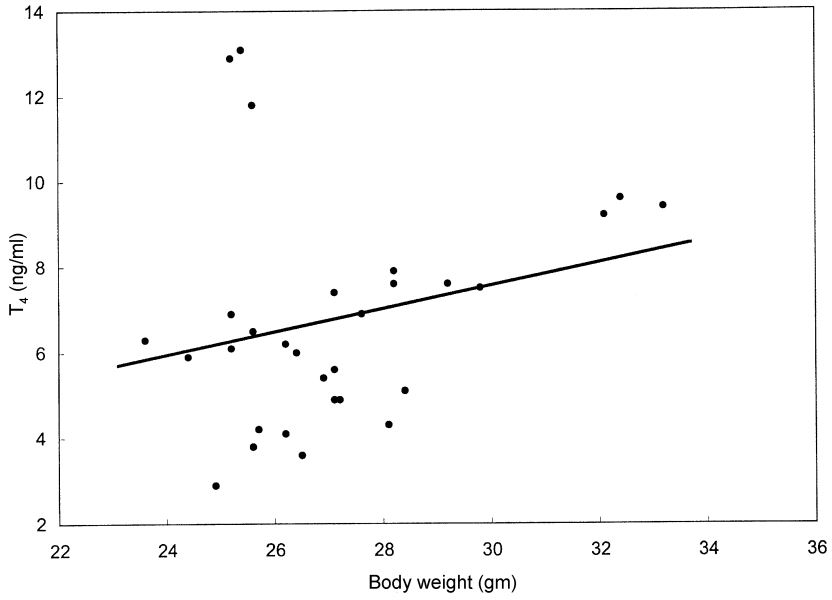

Fig. 4. Correlation between circulating T_4 concentration (ng/ml) with body weight (gm) in female *T. longimanus*

Changes in thyroid microstructure

Thyroid gland showed marked seasonal variation in structure. The follicle cells were low cuboidal; only few were flat and squamous with a centrally placed nuclei. During quiescence, thyroid follicles were homogeneous in appearance, contained small and flattened cells having scanty cytoplasm and small nucleus; some follicles contained small amount of colloid in their lumen (Fig. 5). During recrudescence, in November, thyroid contained heterogeneous population of small to large-size follicles. Large follicles filled with colloid were found at the periphery (Fig. 6). During post-breeding phase in February and March, thyroid comprised only smaller follicles and large lumen filled with colloid. It may seem difficult to reconcile the microstructural changes using the low magnification photomicrographs (Figs 5 and 6), but a detailed microscopic study did reveal the observation described above.

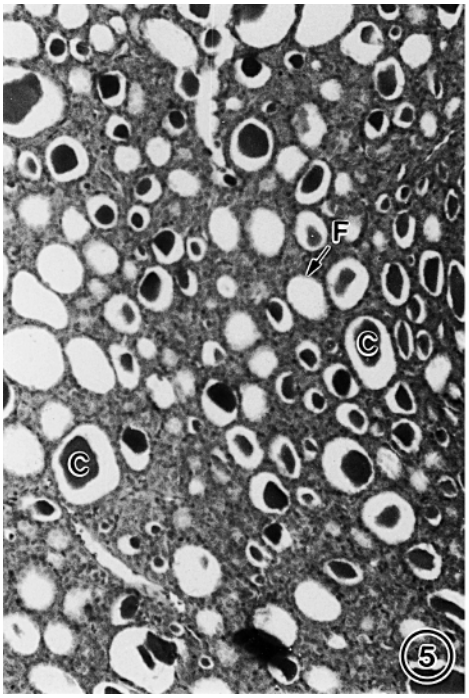

Fig. 5. Thyroid during quiescence showing smaller and empty follicles.
F – follicle; C – colloid

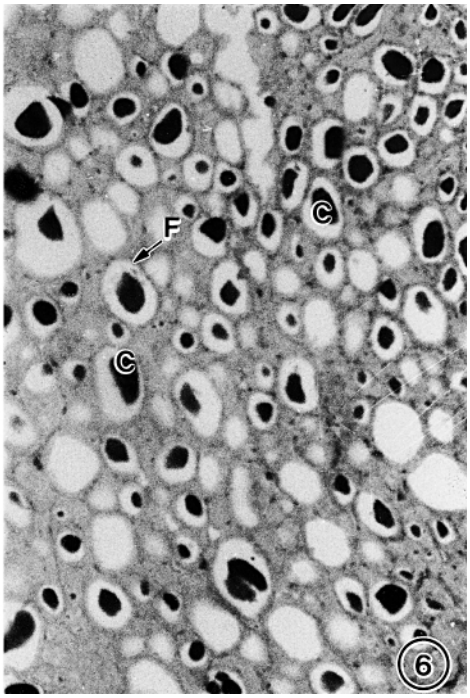

Fig. 6. Thyroid recrudescence showing large follicles filled with colloid.
F – follicle; C – colloid

Table 1
Correlation coefficient between circulating T_3/T_4 concentrations and body, ovary, and thyroid weights in *T. longimanus*

	Correlation coefficient	P values
Quiescence to early-mid-winter dormancy		
Body weight vs T_3/T_4	r = 0.56	P < 0.01
Ovary weight vs T_3/T_4	r = 0.73	P < 0.01
Thyroid weight vs T_3/T_4	r = 0.70	P < 0.01
Body weight vs thyroid weight	r = 0.56	P < 0.05
Ovary weight vs thyroid weight	r = 0.76	P < 0.01
During two successive pregnancies		
Body weight vs T_3/T_4	r = 0.15	NS
Ovary weight vs T_3/T_4	r = 0.21	NS
Thyroid weight vs T_3/T_4	r = 0.23	NS
Body weight vs thyroid weight	r = 0.16	NS
Ovary weight vs thyroid weight	r = 0.14	NS

The data obtained from body, ovary and thyroid weights and circulating T_3 and T_4 concentrations were analyzed by one-way analysis of variance. P value of <0.05 was considered as significant. NS – not significant; r values – regression values.

Table 2
A partial listing of species in which thyroid function has been implicated with reproductive cycles

Species	Observation	Reference
Antrozous pallidus	thyroid active in hibernation	[29]
Tadarida mexicana *Macrotus californicus*	not responsive to ambient temperature of 22–37 °C	[29]
Antrozous pallidus	thyroid active in hibernation through 6th day at 4 °C	[30]
Rhinoliphus hipposideros *Plecotus auritus* *Myotis myotis* (% &)	ultrastructural differences in both follicular and parafollicular cells were seen during hibernation	[34]
Myotis myotis	thyroid in active period showed pronounced changes in the number and size of the organelles	[35]
Macrotus waterhousii (% &)	T_4 low during early pregnancy	[7]
Epomops franqueti (% &)	T_3 low in non-pregnant and T_4 has no change	[15]
Myotis lucifugus (%)	T_4 increased in hibernation	[8]
Scotophilus heathi (%)	T_4 maximum during post-breeding season	[20]
Taphozous longimanus (%)	T_3 and T_4 low during early pregnancy	[32]

DISCUSSION

The present study points out marked seasonal variation in serum T_3 and serum T_4 concentrations. A close relationship between body, ovary and thyroid weight with morphological features of thyroid gland with T_3 and T_4 concentrations was evident from quiescence to early to mid-winter during the reproductive cycle of female *T. longimanus*. This study clearly shows a correlation between body, ovary and thyroid weights from quiescence in August to early to mid-winter dormancy while no correlation was noticed during two successive pregnancies. Histological changes in the thyroid microstructure showing seasonal variation have been reported earlier [20, 34, 35] and therefore, were somewhat de-emphasized in deference to the parameters mentioned above.

The changes in the body weight were usually associated with changes in availability of food and fat accumulation as seen in many other bat species [1, 20, 27]. The body weight of *T. longimanus* increased due to accumulation of fat and adipose tissue prior to winter in November. The fat is metabolized during winter due to scarcity of food [1]. The abundant presence of food and its high intake during August to October was stored as fat in the body. During this period body, ovarian, and thyroid weights along with T_3 and T_4 show correlation with each other. Our observations suggest two phases of body, ovarian and thyroid weight development in which the first phase (August to November) depends on high food intake and the second phase (January to July) depends on low food intake. This nutritional change effects both thyroid and ovarian activity through the hypothalamus-pituitary axis as reported in *Gallus domesticus* [9]. The increase in body weight occurred during November due to high food intake from August to October. The increase in ovarian and thyroid weight during April may be the reflection of a second period of ample food availability during February and March.

Studies on thyroid activity are mainly restricted to temperate-zone bats that exhibit prolonged hibernation [7, 8, 15, 29, 30, 34, 35]. The thyroid activity in these species has been found to be increased in spring and declined in the autumn, remaining quiescent throughout the hibernation period. A slight difference was noticed in *Myotis lucifugus* [8] in which plasma T_4 levels were lowest at the time hibernation began and attained maximum values at the time of arousal. Recently, Krishna and Singh [20] studied thyroid function in relation to reproduction in a vespertilionid male *(Scotophilus heathi)*. In this species, correlation between thyroid and testis weight was not positive during breeding and post-breeding phase. The body weight also did not show any correlation with thyroid weight during breeding to post-breeding periods. The T_4 concentrations were higher in the late winter and suddenly declined to low levels during early stages of pregnancy. The results in *T. longimanus* support the hypothesis that thyroid activity is necessary for transition from breeding to non-breeding phase as in seasonally breeding animals [36].

The T_3 and T_4 concentrations show unique levels during late winter dormancy in *T. longimanus*. The T_3 and T_4 concentrations increased dramatically in late winter. This was similar to hibernating mammals such as *Spermophilus tridecemlineatus*

[14], *Spermophilus richardsoni* [21], *Myotis lucifugus* [8]. The possible explanation for high T_3 and T_4 during late winter dormancy in January in this species is that acute cold may increase the secretion of hypothalamic thyroid releasing hormone (TRH). This increase in TRH enhances the activity of thyroid stimulating hormone (TSH), and ultimately increases the T_3 and T_4 concentrations [3, 24, 33]. The increase in TRH in response to cold might be the reason for the change in T_3 and T_4 concentrations in early stages of first pregnancy in the present study as also reported earlier [28].

The embryonic growth and pregnancy was effected by the level of the thyroid hormone [22, 25, 37]. The effect of thyroid hormone on pregnancy in bats was first reported in *Macrotus californicus* [7]. The maternal thyroid was shut down during early stages of pregnancy in this species. Our present report corroborates the findings in *Macrotus californicus.* The T_3 and T_4 concentrations declined during the early stage of first pregnancy, however no such changes in T_3 and T_4 concentrations were noticed during second pregnancy. This might be a reason for the delayed embryonic development in this species. One possible explanation for differential rate of fetal development in the two successive pregnancies was scant food supply, low environmental temperature and some neuroendocrinological factors during the first pregnancy might result into delayed embryonic development. This conclusion draws support from the previous findings of Bradshaw [5] in *Macrotus californicus* in which the low environmental temperature and food shortage was believed to be responsible for delayed development.

In conclusion, the role of the thyroid in reproduction is more baffling than any other gland studied so far. In *T. longimanus,* a correlation was noticed between body, ovary and thyroid weights with circulating T_3 and T_4 concentrations before winter dormancy in December. However this correlation was not observed during the two successive pregnancies. This indicates a nutritionally dependent ovarian and thyroid activity during this period. This activity might be mediated by thyroid and body weights during this period. The low concentration of T_3 and T_4 during early stages of fetal development in the first pregnancy might be responsible for the differential rate of fetal development in this species. The pattern of thyroid activity noticed in the present study closely resembles the report on other bat species such as *Macrotus* [7, 20]. Our observations further suggest that thyroid function is necessary for transition from breeding to non-breeding state in seasonally breeding animals. This suggestion should be tested further in species, which have breeding cycles similar to *Taphozous longimanus*, as well as in other species, which are monotocous.

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