CHAPTER 2

VARIATIONS IN THE BODY WEIGHT, GONADAL WEIGHT AND GSI WITH RELATION TO THE HORMONAL PROFILE (TESTOSTERONE AND PROGESTERONE) IN JUNGLE BABBLER WITH RESPECT TO ITS REPRODUCTIVE STATE.

INTRODUCTION ~

Because of their instinctive nature, animals tend to breed during that time of the year which is most favorable for the survival of maximum number of young ones (Immelman, 1971; Phillips *et al.,* 1985) hence, the gonadal cycle is generally schedule (particularly timing and duration of the regenerative phase of the cycle) is timed during the period of food abundance, which becomes a characteristic for a species (Naik and Razack, 1967). Thus, gonadal cycle is dependent on the timing and duration of breeding season which in turn is dependent on appropriate environmental stimuli (Nalbandov, 1970; Phillips *et al.,* 1985) and its seasonality to initiate reproductivity and physiological mechanisms for the same (Hau, 2001). The breeding cycles of birds are under the influence of partly exogenous and partly endogenous factors (Naik and Razack, 1967; Lofts and Murton, 1973; Phillips *et al.,* 1985).

Majority of birds in northern hemisphere prefer long summer days for breeding and show distinct breeding and non- breeding seasons through out the year, interrupted by distinct regressive and recrudescent phases. Gonads develop into comparatively voluminous organs during breeding season facilitating gametogenesis as well as steroidogenesis and regress remarkably during the non - breeding season, when the process of gametogenesis and steroidogenesis concludes (Marshall, 1961; Nalbandov, 1970). During this cycle, the concerned hormones are secreted gradually in increasing or decreasing amounts. Reproductive hormones regulate and synchronize all aspects of breeding from the maturation of the sperm and eggs to the expression of the species specific behavioral pattern which are used to defend territory, court a mate and ensure fertilization of the eggs their incubation, hatching and development too. Thus, the changes in the hormonal levels are expected to have varied behavioral effects which are of great reproductive significance (Nalbandov, 1970; Lofts and Murton, 1973; Hau 2001).

Testosterone is responsible for the growth, development and maintenance of the male reproductive system and secondary sexual characters throughout the breeding season. In males, seasonal changes in the testes size and histology have been correlated with reproductive behavior which continues through out the nest building and culminates around the time of egg laying. Sexual displays by males include strutting, aggressive hop-charging and wing-flipping which are closely synchronized with those of the females, and are paralleled by endogenous changes in the reproductive hormones (Johnson, 1986).

In females, the sexual receptivity is controlled by the endogenous secretions of the ovary (mainly estrogen in synergism with progesterone). Progesterone along with testosterone stimulates nest-building in both females and males and also stimulates incubation behavior in both the sexes by stimulating the release of endogenous prolactin from the bird's hypophysis (Balthazart, 1983). Progesterone plays a dual role of a hormonal substance and an important biosynthetic intermediate in the formation of androgens, estrogens and corticoids (Johnson, 1986b).

Voluminous literature exists on the annual breeding cycles of birds. About half a century ago, greater attention has been given to the seasonal breeding cycles of the temperate seasonal breeders with more emphasis on the migratory birds (Marshall, 1961). However information regarding subtropical species is inadequate. Hence, present study deals with the hormonal variation in a common bird of semi arid subtropical region of India- Jungle Babbler. In the present chapter with variations in the levels of testosterone and progesterone, gonadal variations along with the changes in the GSI of the breeding and the non-breeding males and oviducal weight in females are considered.

MATERIAL AND METHODS ~

Jungle Babbler, a common bird of the sub-tropical region of India is known to be irregular breeders. It's a social bird which remains in a flock of 7-8 birds during the non-breeding season while in the breeding period, the flock breaks up into 3-4 birds which includes the breeding parents along with the genetically related helpers which forgo their breeding in order to help the breeding pair in incubation, feeding and taking care of the young ones (Andrews, 1968). Various ornithologists have given different periods of nesting by the Jungle Babblers, from April- August (in Saurastra, Dharmakumarsinghji, 1954), March – September (Whistler, 1949) and irregularly throughout the year (Ali, 1993). Andrews (1968), has reported that the Jungle Babblers breed in and around Baroda District Long 73^0 15′ E Lat 22^0 17′ N and latitude from April – August.

Birds were obtained locally from September 1997′ to July 2000′ and were maintained in an aviary. Food and water were given *ad libitum*. Birds remained in good condition and received unrestricted natural light. Before sacrificing, the birds were weighed individually in an open pan balance. The gonads were removed from the body and freed from the tissue fluid. The weight of the left testis and right testis were recorded separately to the accuracy of 1mg. The male birds with the larger breeding testes were found from April to November while during this period some males were in non – breeding state too.

However, from December to February no males were found with developed/breeding testes. For the convience the males with testicular weight of more than 100mg were pooled together as breeding males and those with less than 30 mg of testicular weight were considered as non-breeding males. The intermediates were not considered for the breeding status as they could be either in pre breeding or the post breeding state as Jungle Babbler is a sub-tropical bird and has a long breeding season. In female Jungle Babblers along with the ovarian weight, the oviducal weight was also recorded. Female Jungle Babblers shows a prolonged breeding phase from March to December when breeding ovary was present. During May, July, August and November, December, maximum numbers of females were in a breeding state with physiologically functional ovaries. However, during these months, fully regressed ovaries were also found. When ovarian weight, oviducal weight and the steroid hormone levels in the female birds were tabulated, a third distinct group different from the breeding and the non-breeding birds could be noted with larger ovary but underdeveloped oviduct. This group was considered as probable helpers for the convience of discussion. No such group could be separated in male birds.

Before sacrificing, blood was collected in the heparinised test tubes from the ventricle of the anesthetized birds and later centrifuged for 60 minutes at 3000 rpm. After centrifugation plasma was collected in Epandorf tubes and stored at -4°C. The separated plasma fraction was used for quantitative measurements of progesterone and testosterone respectively in both male and female Jungle Babblers.

(A) EIAgen progesterone kit:

To evaluate progesterone content in blood plasma a micro Plate solid phase enzyme immunoassay kit was used. The EIAgen progesterone kit contains: a progesterone micro Plate, progesterone calibrators, progesterone conjugate, washing solution, TMB H_2O_2HS, stop solution (H_2SO_4). The solid phase enzyme immunoassay for progesterone is a competitive type immunoassay wherein horseradish- peroxidase-labeled progesterone (HRP-progesterone) competes with the progesterone present in the sample for a fixed and limited number of antibody sites immobilized on the wells of the microstrips. Once, the competitive immunoassay reaction has occurred, the wells are washed and the HRP- progesterone fraction bound to the antibody in the solid phase is measured by adding a chromogen/substrate solution which is converted to a blue compound. After 15 minutes of incubation, the enzyme reaction is stopped with sulphuric acid which also changes the solution to a yellow color. The absorbance of the solution is measured photometrically at 450 nm and is inversely related to the concentration of the progesterone present in the sample. Calculations of the progesterone content in the sample are made by reference to a calibration curve.

(B) EIAgen testosterone kit:

To evaluate testosterone content in blood plasma a micro Plate solid phase enzyme immunoassay kit was used. Principal of the assay is same as for the EIAgen progesterone.

EIAgen testosterone kit contains: testosterone micro Plate, calibrators, and conjugate, washing solution, EIAgen TMB H_2O_2HS and stop solution. The procedure is similar to that for the progesterone with modification in respective microPlate, calibrators and the conjugates.

Calculations of results for progesterone and testosterone:

To calculate the mean absorbance of calibrators and samples (A), the absorbance of the chromogen blank (Ac) is subtracted from the absorbance of all the samples. This is considered as the corrected value. Corrected values of the sample are divided by the corrected absorbance of the zero calibrator (Ao) and multiplied by 100.

A-Ac/ Ao- Ac X 100.

The respective progesterone and testosterone values are plotted on the logit log or semi log graph paper and the concentrations of progesterone and testosterone in the samples are determined by the interpolation from the calibration curves

Table 1: **Body weight, Gonad weight and Gono-somatic index in Breeding and non-breeding males and females and Oviducal weight in female breeding, non-breeding and helper Jungle Babblers (*Turdoides striatus*)**

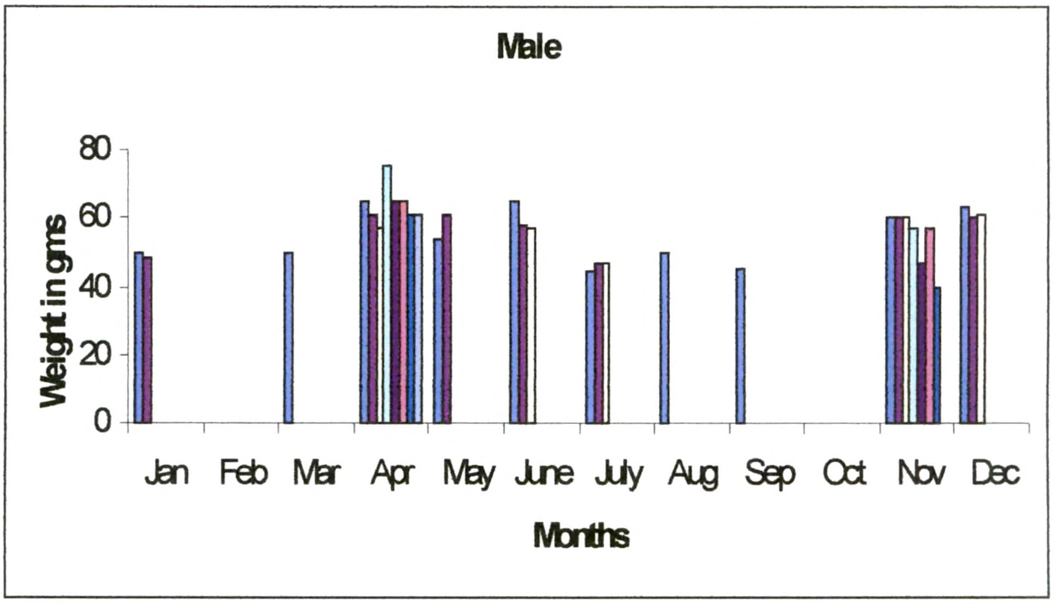
	MALES			FEMALES			
	Body wt (gm)	Testes wt (mg)	GSI	Body wt (gm)	Ovary wt (mg)	Oviduct wt (mg)	GSI
BREEDING	61.4 ±2.27	205.0 ±14.0	0.326 ±0.032	58.66 ±4.50	72.66 ±9.39	181.66 ±30.17	0.129 ±0.024
NON-BREEDING	56.77 ±2.24	13.0 ±1	0.025 ±0.004	54.0 ±2.47	9.0 ±1.27	8.71 ±2.87	0.028 ±0.011
HELPERS	-	-	-	58.14 ±1.86	65.71 ±6.03	47.57 ±8.30	0.113 ±0.010

TABLE 2: Progesterone and Testosterone levels (ng/ml) in blood plasma of male and female Jungle Babblers.

	MALES		**FEMALES**	
	Progesterone	**Testosterone**	**Progesterone**	**Testosterone**
BREEDING	0.3 ± 0.025 *	0.76 ± 0.17 *	1.13 ± 0.066 ***	0.65 ± 0.050 ***
NON-BREEDING	0.15 ± 0.054	0.21 ± 0.040	0.16 ± 0.042	0.18 ± 0.049
HELPERS	-	-	0.7 ± 0.00 ***	0.63 ± 0.033 ***

* $P < 0.05$ ** $P < 0.005$ ***$P < 0.0005$

Figure 1: Body weight of male and female Jungle Babblers during different months



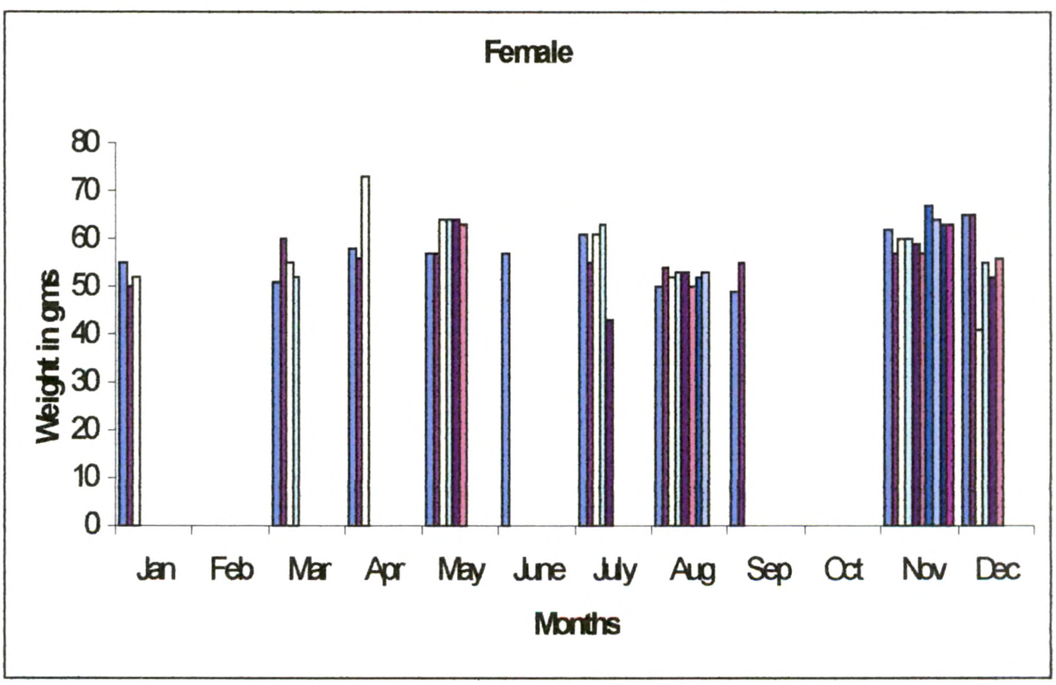
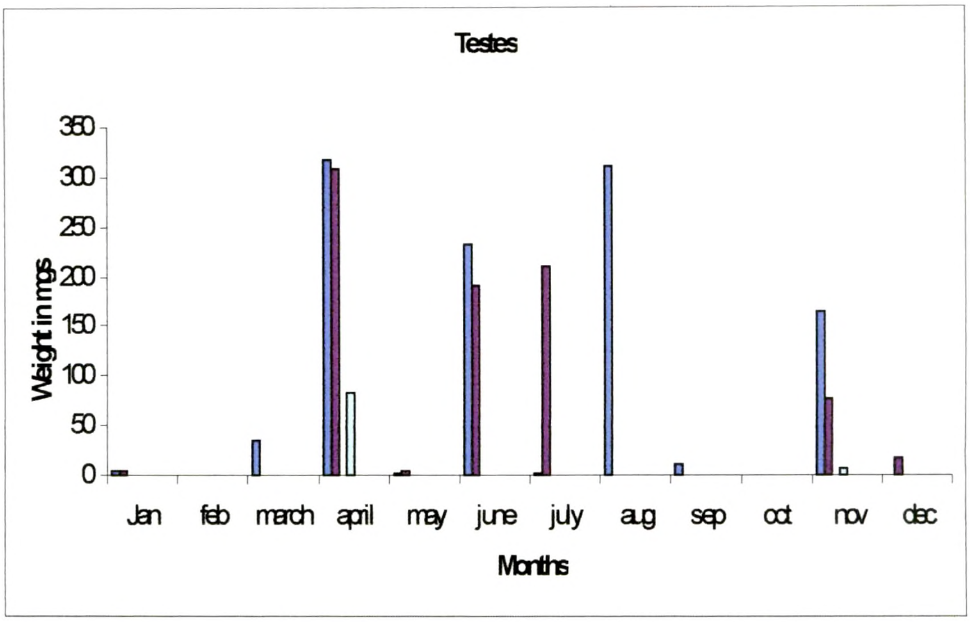


Figure 1a: Gonad weight of male and female Jungle Babblers during different months



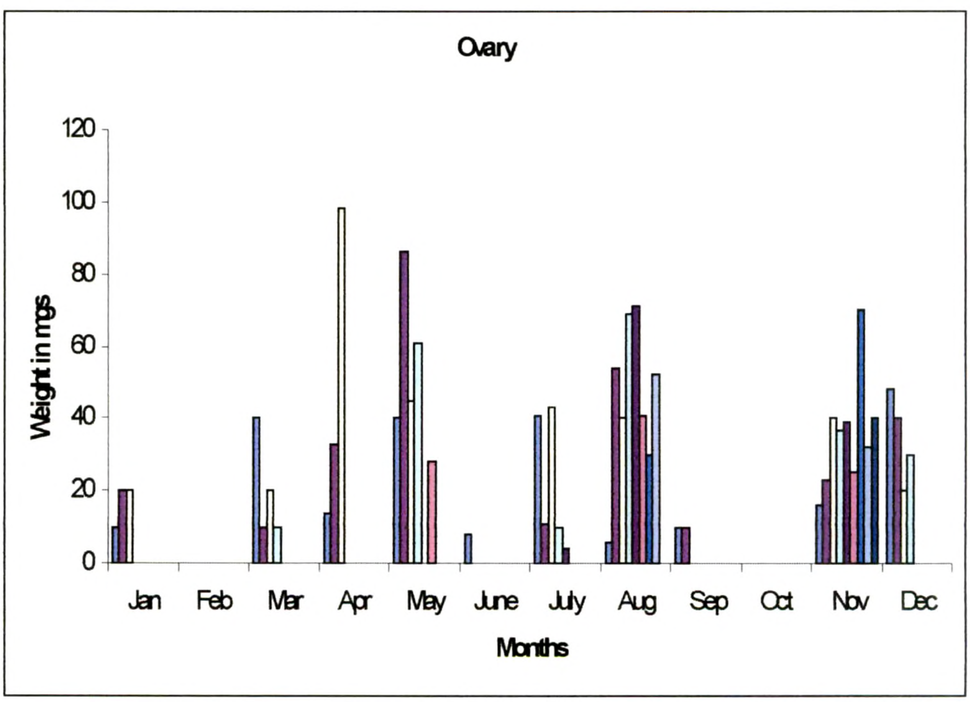
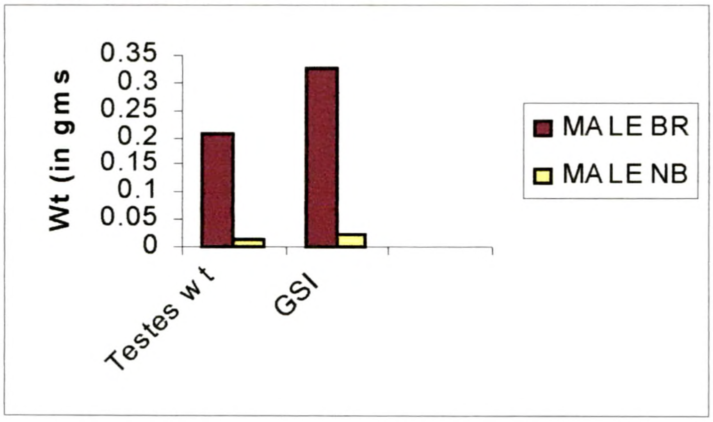
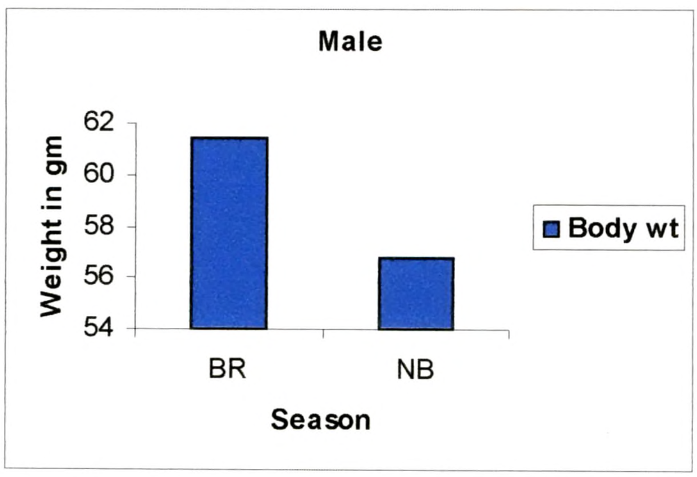


Figure 2: Body weight, Testicular weight, GSI and Progesterone and Testosterone in breeding, non-breeding male Jungle Babblers





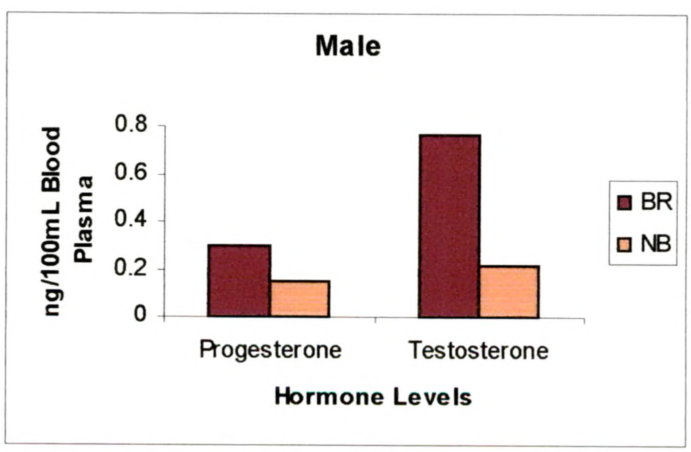
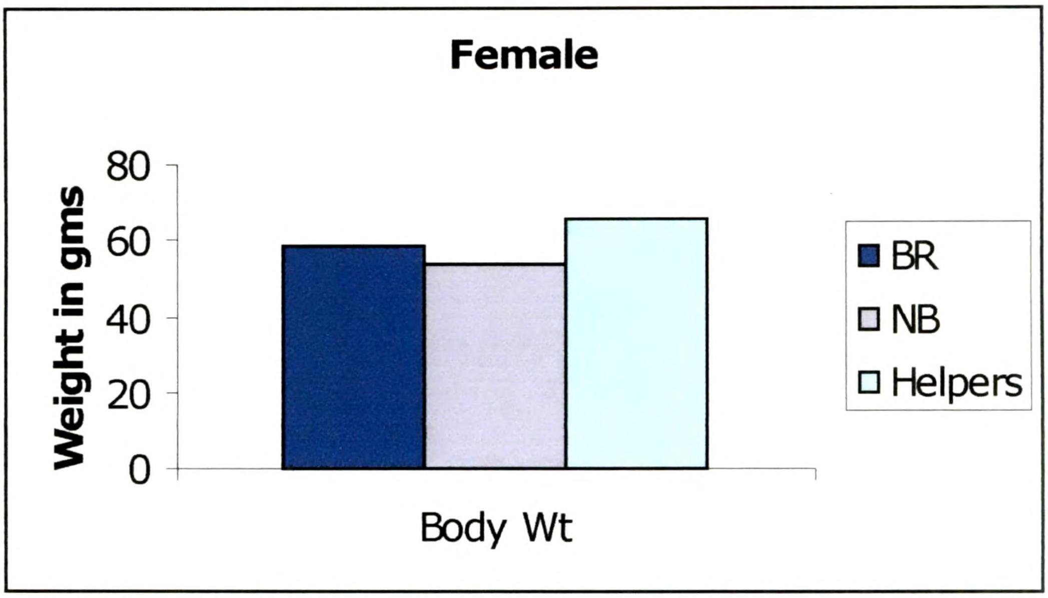


Figure 3: Body weight, Ovarian weight and GSI in breeding, non-breeding and helper female Jungle Babblers



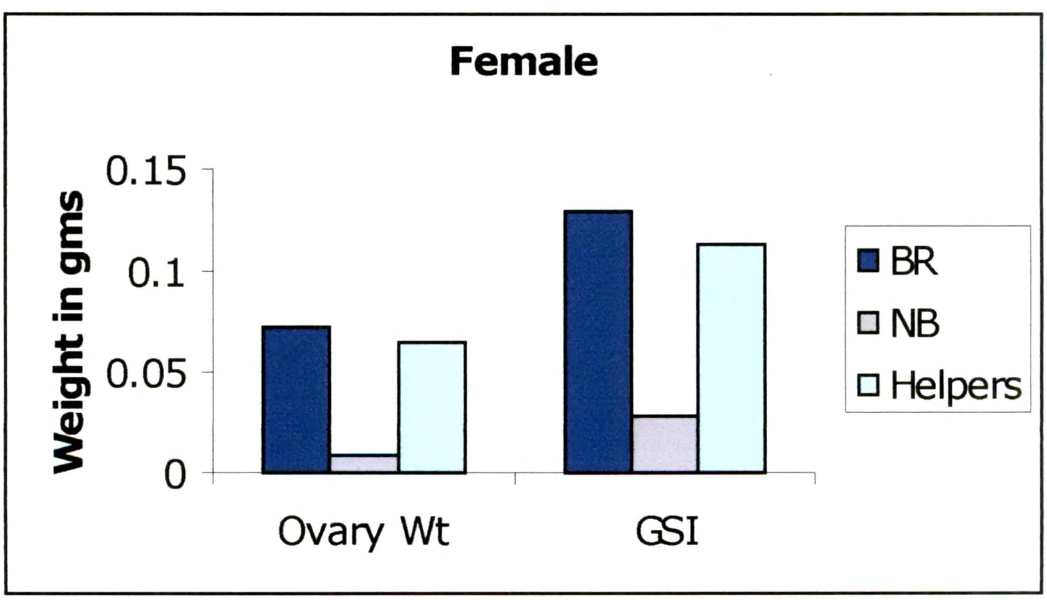
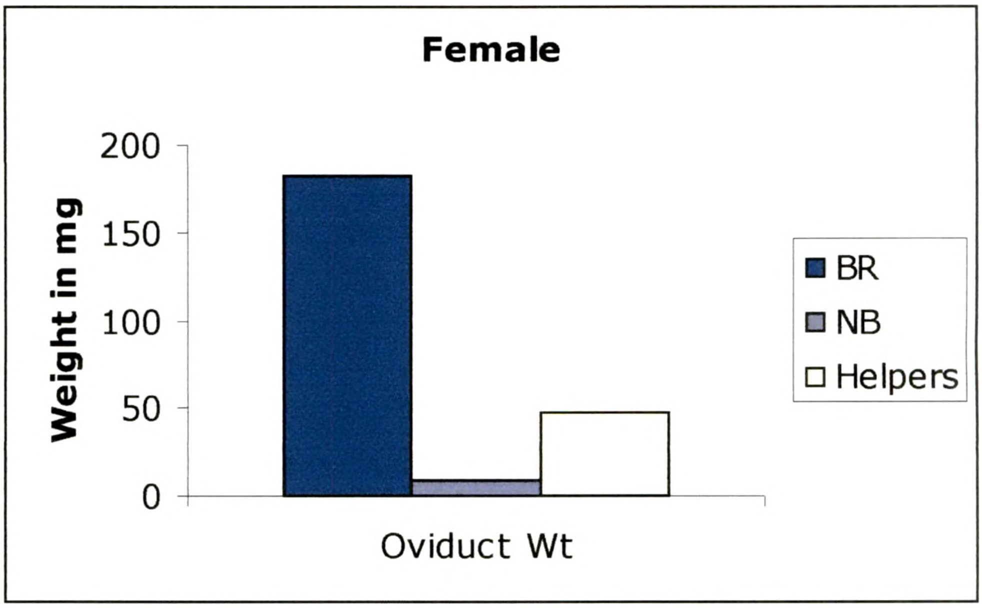
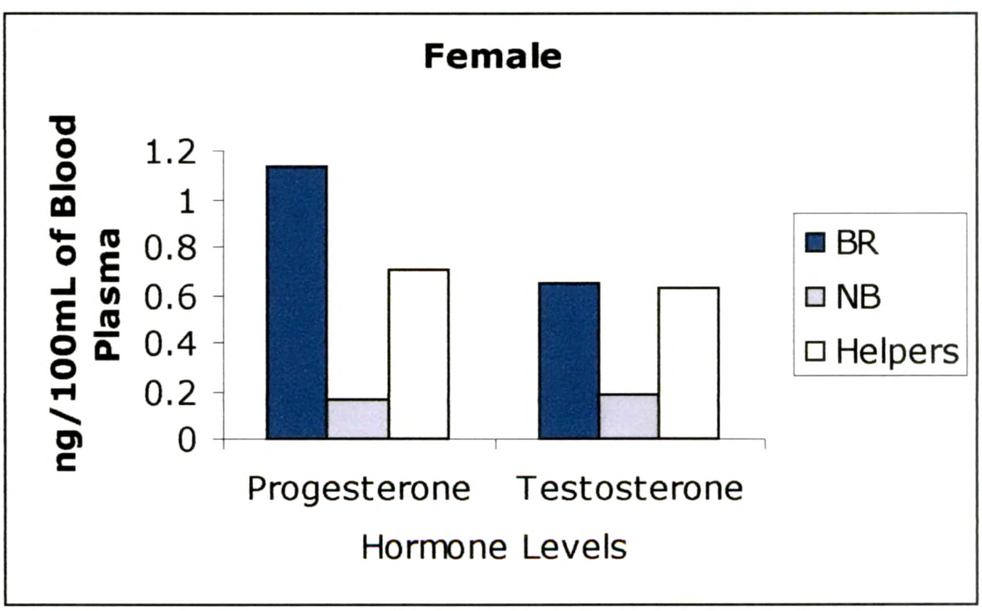


Figure 3a: Hormone levels (Progesterone and Testosterone) and Oviducal weight in Breeding, Non-breeding and helpers female Jungle Babblers





RESULTS ~

As seen in Figure 1 minor parallel fluctuations in the body weight of the male and female Jungle Babblers can be seen. Average body weights were higher during the summer months of March to May as well as winter months of November and December in both the sexes.

When individual gonadal weights are plotted on the graph (Fig. 1a), it can be seen that breeding testes were present from April to November with a drop during September, whereas large breeding ovaries were found from March to December. Females with larger ovary but undeveloped oviducts were found almost all through out the year.

Table 1 shows body weight, testicular weight, ovarian weight and oviducal weight along with GSI for both male and female Jungle Babblers. Progesterone and Testosterone levels for both the sexes are given in Table 2.

MALE:

The average body weight of breeding males was 61.4 $\pm$ 2.27 Gms while that of the non - breeding males was non - significantly low at 56.77 $\pm$ 2.24 gms (Table 1, Fig 2). The mean testicular weight (left and right testis combined) in the breeding males was 205.00 $\pm$ 14 mg and in the non - breeding males was 13.00 $\pm$ 1 mg (Table 1, Fig 2). The mean gono-somatic index for the breeding males was 0.32 $\pm$

0.03 and for the non - breeding males it was 0.02 ± 0.004 (Table 1, Fig 2).

The testosterone levels in the breeding and the non - breeding males were 0.76 ± 0.17 ng/ml of blood plasma and 0.21 ± 0.040 ng/ml of blood plasma respectively (Table 2, Fig 2) whereas the progesterone levels were 0.3 ± 0.025 ng/ml of blood plasma and 0.15 ± 0.054 ng/ml of blood plasma in the breeding and the non - breeding males respectively (Table 2, Fig 2).

FEMALE:

There was no difference in the mean body weight of breeding and the helper females at 58.66 ± 4.5 gms and 58.14 ± 1.86 gms respectively. In non-breeding females the body weight was non-significantly lower at 54.0 ± 2.47 gms (Table 1, Fig. 3). The average weight of the ovaries of the breeding females was 72.66 ± 9.39 mg which was significantly higher than the non - breeding females at 9.0 ± 1.27 mg, while in helper females the ovary weight was 65.71 ± 6.03 mg (Table 1, Fig 3). The weight of the oviduct in breeding females was 181.66 ± 30.17 mg whereas it was significantly low in non - breeding females at 8.71 ± 2.87 mg, while in case of helper females it was maintained at 47.57 ± 8.30 mg (Table 1, Fig. 3a). The mean gono - somatic index of breeding females was 0.129 ± 0.024 while that for the non - breeding females was 0.028 ± 0.011 and in helper females it was 0.113 ± 0.010 (Table 1, Fig. 3).

The testosterone levels in the breeding and the non - breeding females were 0.65 $\pm$ 0.050 ng/ml of blood plasma and 0.18 $\pm$ 0.049 ng/ml of blood plasma respectively; while in the helper females it was 0.63 $\pm$ 0.033 ng/ml of blood plasma (Table 2, Fig 3a). The other hormone progesterone was 1.13 $\pm$ 0.066 ng/ml of blood plasma and 0.16 $\pm$ 0.042 ng/ml of blood plasma in the breeding and the non - breeding females respectively. In helper females it was 0.7 $\pm$ 0 ng/ml of blood plasma (Table 2, Fig 3a).

DISCUSSION ~

Diurnal rhythms in hormone – dependent characteristics, including behavior are a ubiquitous feature of avian and mammalian reproduction. Such rhythms could arise from diurnal rhythms in the sensitivity of the target organs to hormonal stimulation. The behavior is probably programmed by the animal's genetic constitution and modified by the environment and hormonal status. Exposure to hormones in the mature birds generally elicits specific behavioral patterns (*e.g.* reproductive behavior). An adaptation of birds to seasonally changing environment implies alterations in the physiology, including changes in the endocrine system. The field endocrine studies have greatly expanded the knowledge of how hormones interact with social and ecological factors to affect the timing, sequence and frequency of reproductive activities (Morton *et al.,* 1990). It is well established fact that androgens and their metabolites play an essential role in the induction and maintenance of aggressive and reproductive behavior in birds. Both estrogen and androgens are involved in the regulation of aggressive nesting, courtship and copulatory behavior during the different stages of reproductive cycle.

The gonads of Jungle Babbler undergo changes characteristic to the avian species in weight and functions over the breeding cycle. As seen in fig 1a the breeding birds were found from April to November leaving other months for the gonadal regression mainly during December to February. According to Andrews (1968), the adult males are in readiness to breed between April to November, irrespective of whether the male actually breeds or not. The testes in Jungle Babblers seem to regress fully only for a short period indicating that an adult male is capable of breeding more than once in a course of the long breeding readiness, whether it actually breeds or not depends upon its success in acquiring a mate that is in readiness to breed. As seen in other northern hemisphere birds (Farner and King, 1973; Philips *et al.*, 1985), Jungle Babblers too probably initiate their breeding on the onset of longer summer days and have a longer breeding cycle when some pairs are ready to breed and a very short non-breeding season from November to February.

Cyclic changes in the weight of the resident species are reported to reflect the seasonal changes in the feeding conditions. The diet of Jungle Babblers though mainly insect varies from the graminivore type to insectivore and vice- versa depending on the availability of food (Gaston, 1978). Jungle Babblers are known to be chiefly insectivores (during the kharif season) in the pigeon pea fields infested with *Helicoverpa armigera* (Gokhale, 1992; Parasharya, 1988). While during the Rabi season when the pulses and cereals like Bajra, sorghum and pearl millet are sown, Jungle Babblers are known to feed on these grains (Parasharya, 1988; Gaston, 1978). It is marked that some species gain in weight during breeding while in others the body weight does not fluctuate. In the present study, the two sexes differ in their body weights non- significantly. Breeding males weighed heavier

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than the breeding females, while the weight loss of the males and females was almost non-significant during the non-breeding state. Helper females maintain the weight to that of the breeding females. It was not possible to separate helper males from the breeding and the non – breeding males. The variation in the mean body weight does not show any marked variation in relation to breeding activities and as mentioned earlier can be correlated with the nature of the Jungle Babblers' habitat and feeding habit. In case of the Jungle Babblers the energy expenditure on the vital activities like moulting and reproduction is distributed over a longer period of time by the bird (Andrews, 1968). He has related the increase in the mean body weight from April to August to one or both of the following factors operative during these months (i) availability of relatively numerous food items (ii) intense foraging activities during the longer summer days.

As seen in Table 1, there is no difference in the gonadal weights of both breeding and helper females indicating that there is no relation between the body weight and gonadal development. The Jungle Babblers are co-operatively breeding birds, where some individuals called helpers forgo their breeding in order to help the breeding pair in raising the young one. The helpers in the community are genetically related to the breeding pair and are usually the offspring from a previous nesting attempt (Andrews, 1968; Philips *et al.,* 1985). When the energy costing activities like incubating the eggs and feeding the young ones are shared not only by the parents but by all the members of the flock, no predeposition of fat will be required especially in case of females, and thereby their body weight and that of the helper females probably remain same during the breeding state. Also Jungle Babbler females have a very long breeding readiness preventing them to show distinct increase in the body weight. The GSI of helper females is also almost equal to that of the breeding females. Though there is no difference in the ovarian weight and body weight of breeding females and helpers, a remarkable difference is seen in the oviducal weight probably under the influence of sub-dued production of progesterone from the ovary retarding the full growth of the oviduct. In these groups of birds *i.e.* helper females, the progesterone level were intermediate to those observed in the breeding females and the non – breeding females (Table 1).

Sexual behavior is dependent on the synthesis and release of gonadal steroids *viz.* estrogens and progesterone in the females and testosterone in males. Plasma levels of testosterone and progesterone determined in the breeding and the non – breeding individuals as well as helper females in Jungle Babblers, an altricial passerine with nearly continuous/long reproductive readiness indicate that reproductive behavior including incubation and feeding is shared almost equally by both the parents or rather by the members of the whole flock (helpers). The gonadal development of these birds initiated in March and continued till November, which is reflected with the increase in the circulating testosterone and progesterone levels in both the sexes as well as in helpers. In males, different components of sexual behavior are facilitated by direct and indirect action of testosterone. In social bird Mexican jays (*Aphelocoma ultramarine)*, Vleck and Brown (1999) have reported elevated testosterone during male-male competition for mate which decreases during parental or alloparental care. Jungle Babbler though have feeding territory (Andrews, 1968) do not show nesting territory and all the individuals of the flock take part in parental care. This is reflected in breeding males having non-significantly high testosterone levels compared to females in breeding state. The helper females also showed plasma testosterone almost equal to breeding females indicating their equal role in breeding activities except for egg-laying. Testosterone has its fitness costs and influences aggressiveness and decreases the tendency to display parental care, hence testosterone is lower when necessity for parental care increases (Vleck & Brown, 1999). An elevated testosterone level in females of Macaroni and Gentoo penguins during post-copulation period has been associated with both sexes being involved in the nest defense (Williams, 1992). Trainor and Marler (2001), observed that in contrast to seasonally breeding birds, testosterone maintains parental behavior in monogamous California mouse (*Peromyscus californicus*) by aromatizing testosterone to estrogen in brain. Testosterone mediates a negative correlation between parental behavior and aggression in several seasonally breeding mammalian species (Trainor and Marler, 2001). This could be true for birds too. In the Bank Myna

(*Acridotheres ginginianus*) a colonial nester- where all the males of the colony are involved in colony defense, rise and fall in the testosterone levels occur sharply from non-breeding to breeding to non – breeding phases whereas in the related species of the same family Sturnidae, Brahminy myna (*Sturnus pagodarum*) - which is an individual hole-nester, the rise and fall in the testosterone levels has been reported in our laboratory to occur gradually (Sapna, 2002). This also indicates the influence of levels of testosterone in aggressive behavior of birds. In Jungle Babblers, where whole flock is involved in defending a single nest, lower titers of testosterone compared to other birds are noted. Female birds are generally less aggressive than their male species which is probably related mainly to the lower androgen production of the ovary compared to the testes. In Jungle Babblers non-significant differences in the plasma testosterone levels are observed in the breeding males and breeding females as well as in the helper females suggesting that the nest defense, taking care of eggs, incubating them and protecting the young ones is a combined flock activity. Testosterone in male birds is known to stimulate nest building activity and the progesterone in females, to stimulate incubation behavior by stimulating the release of endogenous prolactin from the bird's hypophysis (Seiler *et al.,* 1992).

An increase in progesterone secretion has been claimed to play a significant role in mediating the transition from courtship to incubation behavior in female ring doves (Silver *et al.,* 1974). Logan and Wingfield (1995) linked the progesterone rise to breeding behavior in male and female mocking birds (*Mimus polyglottos*). In ovarectomised ring doves' administration of progesterone and estrogen facilitated nest-building and incubation in response to the presence of nesting material and eggs respectively (Cheng & Silver, 1975). However, an average constant basal plasma progesterone level over the different phases of the reproductive cycle in males have been reported in several species of birds like Ring dove, *Streptopelia risoria* (Silver *et al.,* 1974), Chicken (Furr, 1973), Pigeon (Haase *et al.,* 1976), Mallard duck (Donham, 1979), White crowned sparrows (Farner and McCreery, 1979) and Mynas (Sapna, 2002).

In the present study, moderately increased levels of progesterone in males can be related to its social breeding habits where all the individuals of the flock are involved in incubation. Male Ringed Doves treated with Progesterone have been reported to show nest related pre-incubation behavioral patterns depending on experience (Cheng, 1977; Michel, 1977). There is a positive correlation between progesterone concentration and follicular development (Silver *et al.,* 1974). In females, the seasonal variation of progesterone is synchronous with the laying activity. In helper females intermediate levels of progesterone *i.e.* higher than breeding males' but lower than breeding females supports their role in the parental activities. Oviduct development is influenced by progesterone which induces the development of tubular glands in the magnum region of the oviduct

(Hutchison, 1975). It also results in defeathering and increased vascularity of brood patch in the breeding females (Hutchison, 1975). In Jungle Babbler the plasma progesterone showed a pattern of variation different from that of other hormones. Williams (1992), has reported that in both species and sexes of penguins, elevated plasma levels of progesterone occurred between arrival and early chick - rearing. The intermediate progesterone levels in helpers with subdued oviducal development thus indicate the involvement of helper in various reproductive activities except egg laying.

CONCLUSION ~

In tropical birds, different ecological groups in an area may breed at quite different times of the year. As a consequence, some breeding activity may be seen through out the year or at least spread widely over the year, therefore it appears to be difficult to draw any conclusions about the breeding seasons of such birds. In case of Jungle Babblers, even though they are said to be irregular breeders, in a particular zone or area where there is an abundance of food during a specific time of the year, they show peak in the reproductive activities thereby reflecting nearly seasonal breeding pattern.

According to the present investigation, in Jungle Babblers, the gonads show maturity (*i.e.* Gonad weight is maximum) between the months of April to November, suggesting that the Jungle Babblers show more or less seasonal breeding with an extensively long period around the Baroda district.

Babblers are known to exhibit co-operative breeding activities. In the co-operative breeding, the helpers help in many breeding activities including the search for the food and than feeding the young ones. This reflects that the burden of energy expenditure is shared equally by all the individuals in a group and that they rely on daily food supply rather than storing the food. Therefore the physiological status of the pair which is in breeding state is probably nearly similar to that of the individuals in the group which are in non -breeding state and hence they do not show significant differences in body weight during breeding and non-breeding state. Further, when hormonal profiles are compared, the helper shows a condition between breeding and non-breeding females.