ENVIRONMENTAL CORRELATES OF REPRODUCTIVE FUNCTIONS IN RED-VENTED BULBUL (*Pycnonotus cafer stanfordi* Deignan) IN LUMAMI, ZUNHEBOTO DISTRICT, NAGALAND

by

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A Pair of Red-Vented Bulbul

DECLARATION

I, Neelakshi Verma, hereby declare that the subject matter of my thesis is the record of the work done by me, that the contents of this did not form the basis of award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the Nagaland University for the degree of Doctor of Philosophy in Zoology.

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CERTIFICATE

This is to certify that the thesis entitled "Environmental Coorelates of Reproductive Functions in Red-Vented Bulbul (*Pycnonotus cafer stanfordi* Deignan) in Lumami, Zunhheboto District, Nagaland" is a record of original research work done by Ms Neelakshi Verma under my guidance and supervision. She is a registered Research Scholar (Regd No. PhD/ZOO/00128) of the Department.

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INTRODUCTION OF THE SUBJECT ANIMAL

Red-vented bulbul, *Pycnonotus cafer* is a non-migratory finch which belongs to the family *Pycnonotidae*. This avian family incorporates 138 species of bulbuls described under 27 Genera. The Genus *Pycnonotus* comprises of 47 species. The bird is a native of south-east Asia but has established itself in as many as 37 continental locations outside its native range. At present, there are 8 sub-species of *P. cafer, of which Pycnonotus cafer stanfordi* has its distribution restricted to parts of North-East Indian states: southern part of Assam, Arunachal Pradesh, Nagaland, Manipur, Meghalaya, Tripura, Bangladesh, northern Myanmar and extending up to south-western parts of Chinese provinces between 23^{o}N-29^{o}N (ZSI, 2006). The sub-species *Pycnonotus cafer stanfordi* has partially crested black head, scale like markings on breast and back, white rump, and a conspicuous crimson patch below the root of the tail. The bird is well known for its joyous call note, vivacious disposition, and fighting qualities. The adults eat fruits, peas, berries, insects and flower nectar, the young feed on insects and their larvae only.

(Neelakshi Verma)

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“To speak gratitude is courteous and pleasant, to enact gratitude is generous and noble, but to live gratitude is to touch Heaven.” – **Johannes A. Gaertner**

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PREFACE

"There is nothing in which the birds differ more from man than the way in which they can build and yet leave a landscape as it was before."
Robert Lynd

Ornithology is a branch of Zoology that deals with the scientific study of avian species. Feathered bio-pods as bio-indicators are known to paint vivid picture of productivity status, biodiversity load and the carrying capacity of the habitat and thus the general health of the environment. This class of vertebrates exhibit maximum adaptive radiations. Closely related species and occasionally intra-specific requirements and expression of different behavioural attributes may vary depending upon their latitudinal dispersal and dwellings. Further, as shown for many other animals, birds do not exhibit a perfect biological 'Clock' or 'Calendar,' hence they tend to handle their seasonal/annual physiological requirements keeping them synchronized with such physical factors which have maximum precision at their appearance year after the year, although in some species, inbuilt mechanisms continue operating to fine tune their reproductive seasonality tied with the presence of species-specific quality/quantity food resources in the environment.

Amongst environmental variables, seasonal changes in natural lighting hours by virtue of their absolute latitudinal precision have potential to herald the approach of propitious timing for breeding season and accordingly, trigger the cascade of events leading to the onset of seasonally important physiological events like, reproduction, moult and migration. Internally, thyroid glands have been shown to provide a connect between environment and the neuroendocrine mechanisms to effect temporal adjustments into body physiology to manifest expression of different behavioral attributes like, courtship, pair-bonding, nest-building, territoriality, mating, egg-laying, incubation, fledging and parental care *etc*. followed by plumage renewal and migration to happen in quick succession. Further, the efficacy of environmental variables on the neuroendocrine system is likely to differ from equatorial region towards the pole. Progressive increase in daylengths away from equatorial region might impact physiological attributes to cast mandatory trade-off across species to enhance their survival fitness. It would indeed be interesting to decipher how far these features influence latitudinal distribution of species/sub-species?

During the past few decades several reviews have been made to detail the impact of environmental variables in the integration of neuroendocrine functions during culmination of avian breeding cycles, and in the process, role of daylength appears to have been over emphasized cornering contribution of edaphic and other environmental variables like temperature, humidity and rainfall which might play pivotal role during the expression of breeding cycles. However, it is now generally conceived view that daylength by virtue of its potential in driving and/or fine tuning inherent physiological rhythms help culmination of reproduction in some or more species at all latitudes and possibly in all habitats intimately tied with availability of food resources in the habitat. Many species have been shown to exhibit well defined circadian and/or circannual photoperiodic response system to aid the development and termination of breeding season and expression of associated physiological and behavioural attributes. Recent developments in the field of avian reproductive physiology entail functional role of thyroid hormones during the development and termination of photoperiodic response in birds by selective switchover to concurrent up-regulation of type-II and type-III deiodinase genes and consequential change in anatomical features of neurons involved in the synthesis and secretion of gonadotrophin releasing hormones in the mediobasalhypothalamus. The concept suffers from differences in fact and the opinion and hence warrants for many more investigations involving species from different latitudes and possibly from different habitats.

In the present dissertation efforts have been made to study: (i) Seasonality in gonad and body mass regulating mechanisms, food and feeding ecology and expression of different aspects of physiological and behavioral attributes. (ii) Seasonality in photoperiodic response system and (iii) Role of thyroid in the development and termination of breeding cycles of red-vented bulbul, *Pycnonotus cafer stanfordi*.

"God loved the birds and invented Trees, Man loved the birds and invented Cages."
Jacques Deval

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CHAPTER – I

GENERAL INTRODUCTION

Earth's rotation at 23.5° inclined axis yields daily light-dark cycles and its revolving around the Sun in an elliptical orbit leads to the evolution of seasons. By virtue of their unrelenting mode of operations, these features acquire a rhythmic fashion. Light, temperature, humidity, rainfall and many more physical features vary in tune with each cyclical change which cast profound influence on the living beings. Living organisms in order to maximize their survival fitness tend to evolve such behavioural attributes which elevate their adaptability levels to stand in tune with natural rhythms. Presumptive adaptations entail gross root preparations which influence or modify different physiological characteristics of the organisms including their metabolic, physiologic and behavioural aspects. With all these features in place, organisms tend to capitalize on predictive environmental information to effect temporal adjustments in such physiological and behavioural attributes which may take time to develop but need to mature synchronized with the presence of optimal environmental conditions (Baker, 1938; Marshall, 1961; Farner and Follett, 1979; Assenmacher and Jallageas, 1980; Thapliyal and Gupta, 1989; Dawson, 2008, 2015; Xiao *et al*, 2017; Kang, 2021).

Essential features of a breeding season in avian forms entail the production of viable eggs, incubation and successful rearing of chicks which place extra calorie requirements that can be met only during certain part of the year when food resources in the habitat ameliorate to accommodate quality food requirements of tender fledglings to help foster their growth and replete energy stocks of adults depleted during intense breeding activities. Seasonal abundance of species-specific food resources in the wild, has therefore, been regarded as the 'ultimate factor' which helps in determining the most propitious time of the year during which young ones can be reared with ease. However, since food resources across habitats flourish linked with dynamic components of the ecosystem, it may not be possible for birds to precisely guess at the timing for the presence of quality food resources in the habitat and therefore, timing breeding season with precision becomes a daunting task. Secondly, putting this feature in place, birds will not be able to find sufficient space to take mandatory preparations for supporting the requisites of potential breeding season. For this reason, natural selection has favoured a switch over to the use of 'proximate factors' which are relatively much more dependable and may thus be used to predict the approach of time conducive for scheduling reproductive season (Baker, 1938; Immelman, 1971; Sharp, 1984a,b; Dawson, 2015).

Food and Feeding Habits

Ornithological studies have paved way to develop foraging theory (Tinbergen, *et al*, 1967; Krebs *et al*, 1977; Zach and Falls, 1977). However, foraging behaviour of a large majority of avian species is yet to be deciphered owing to inherent difficulties which a researcher often confronts in negotiating with long-term observations without missing salient features of breeding strategy. The main food items of birds can be ascertained only by direct observations on variety of food items they consume. Notionally, main diet comprises of at least 60% of the food variety that are savoured by the species (Khan and Naher, 2009). Studies involving different species of birds depending on animal food resources have shown that their distribution in the region of an open area corresponds with the abundance of prey and the extent of conglomeration considerably associated with the abundance of prey, although it has most convincingly been demonstrated only in swallows (Fujita and Higuchi, 2005). Mostly, swallows prey on a variety of flying insects from Hymenoptera, Coleoptera, Hemiptera and Diptera groups while on their wings (Turner, 2004). Generally, they prefer larger insects instead of smaller ones (McCarty and Winkler, 1999). Further, the rate of predation corresponds to the abundance of prey and the ease of their capture (Hespenheide, 1975). The food consumed in different species of swallows varies from insects to fruits and different parts of plants, *for example*, the greater striped swallow, *Hirunndo cucullata* of Africa feeds mainly on seeds of Acasia (Brown and Brown, 1986; Underhill and Hofmeyr, 2007). The storey is likely to keep rolling with wrapping of many more species from different eco-habitats and a wide spectrum of food and feeding choices.

Environment and Breeding Cycles

Current status of literature on avian reproduction describe that breeding in birds is induced by a complex of proximate factors which can be assigned to four categories (Wingfield, 1980, 1983; Wingfield *et al*, 1992). The first which provides initial predictive information is concerned with the initiation and maintenance of physiological condition in which nesting can begin. Successful breeding however, depends on three other categories of proximate factors which initiate final stages of gonadal maturation, synchronise reproductive cycles of mates and otherwise modify the final reproductive effort. The efficacy of proximate factors in influencing avian reproductive system is however, subject to great variation amongst species and even within individuals of a species occupying different habitats (Lack, 1968; Immelman, 1971; Murton and Westwood, 1977; Dawson, 2008, 2015).

Reproductive success of a species has prime bearing on the degree of preparedness which the mates take before stepping into breeding season. In monogamous species breeding is seasonal which begins with the onset of courtship display to facilitate the selection of mate and strengthening of pair-bonding. Further, depending on the competence level of the male partner, pair-bonding may change in the following breeding season or may continue rolling on to the successive breeding seasons (Cézilly *et al*, 2000; Sánchez-Macouzet *et al*, 2013). In some avian species like swans, bald eagles, California condors, and the Atlantic puffins, pair-bonding may be retained for long and may even continue as life-long engagements (Black *et al*, 1996; Ens *et al*, 1996). Further, in certain species, pair-bond retention has intimate bearing on socially dominant males who gain access to females of their choice and thus become social father for the off-springs reared during the season (Slagsvold and Lifjeld, 1994). The gamut of developments enthralling pair-bonding in birds is rather too intricate and subject to profound inter and intra-specific variations.

The significant stage in the breeding cycles of altricial species is vested in pairs' mutual consent about the site for placing their nest and the sentinel behaviour discharged during the course of successful fledging. This forms the most crucial step in the breeding ecology of birds for reasons that it has direct bearing on security concerns of eggs, nestlings and adults and is thus linked to the breeding success and perpetuation of the progeny. The overall scenario seeks presence of features which help ensure successful hoisting of the progeny. Therefore, topographic, climatic and psychic aspects delimit the nest-site selection through input on nest-building resources, surroundings and biotic and abiotic components of the environment (Bhardwaj *et al*, 2006; Asokan *et al*, 2008). Although, features necessitating species-specific requirements may change in accordance with the topography of land and the circumambient environment, four features that seem to constitute the core of all habitats are: (i) an exposed area for foraging (ii) availability of nesting materials in the immediate vicinity and potential sites for camouflaging (iii) drinking water resource in surroundings and (iv) less anthropogenic and predation pressures in and around the habitat.

Nest-Types and Duration of Nest-Construction

Campbell and Lack (1985) have broadly categorised avian nests into different types basing largely on their shape and size which can be detailed as under:

No nest : Some birds do not use nest as a shelter, instead they opt for safer sites to deposit eggs on the breeding ground (Ehrlich *et al*, 1994). Eggs of such birds are edged at one end which prevents their rolling down the ledges as there are no bounds of the nests. Auks, penguins, pigeon guillemot, Eurasian eagle-owl and tawny owl are some of the birds to represent this category.

Scrape Nest: These are shallow depressions made in the soil or amidst plants (Campbell and Lack, 1985) with deep edge to prevent eggs from turning down. Nests are sheathed with small pieces of plants, stones, shells or feathers to provide insulation, position the eggs and prevent them from dipping down in the soil (Ehrlich *et al*, 1994). Ostriches, ducks, shore birds, falcons, pheasants, quails, partridges and bustards belong to this category.

Burrow Nest: The nesting birds dredge a horizontal burrow in the soil and create a chamber at the end of the tunnel to deposit eggs (Ehrlich *et al*, 1994). The length of the tunnel and the substrate used have been found to vary in parakeet (Juniper and Parr, 2003), sand-martins (Ehrlich *et al*, 1994), puffbirds (Rasmussen and Collar, 2002) and barbet (Short and Home, 2002). Puffins, shearwarers, todies, kingfishers, crab plover, miners and leaftossers belong to this category.

Cavity Nest: The cavity nest is made by certain species in live or dead tree trunks and may vary in the size and shape according to the latitude and the clutch sizes (Collar, 2001; Reed, 2001). This forms the safest nest from the view point of protection from the predators and the rain. Woodpeckers, barbets, parrots, tits, blue birds, hornbills, some kingfishers and the owls are the representatives of this category.

Cup Nest: The cup shape nest is smooth, round or semi-circular with a smooth cavity which holds the eggs. Mainly flexible materials, such as grasses, mud or saliva are used in building the nest (Hansell, 2000). In some cases, elastic materials are used to facilitate nests to be easily shaped and fastened (Ehrlich *et al,* 1994; Erickson, 2008). Many passerines and few species of humming birds employ viscous, instant dehydrating saliva to glue their nests to the substrate.

Enclosed nests: This type of nest is constructed by chiffchaff, magpie and autumn-breeding munia species. The basic structure is cup type with extended sides, a roof and an entrance hole not easily noticeable. The hammerkop builds the most bulky and magnificent dome-shaped nests.

Saucer or plate Nest: The appearance of saucer or plate nest is outwardly similar to the cup nest. The size of such a nest is larger as compared to the size of bird which has made it. Owing to the habitat of the birds, such nests can be positioned on the land or floating in water (Czechura and Czechura, 1994; Hyde, 2004). Grebes, coots, black-terns are the representatives in this group of birds.

Pendant Nest: The pendant nests are in the form of lengthened pouch like structure knitted together with flexible materials, such as grass and plants fibres. They are made to hang from the bough of the tree (Davis, 1985), edges of the houses, thorny tree-twigs, telephone wires *etc* (Venkataramani, 1981; Subramanya, 1982; Borkar and Komarpant, 2003). Oropendolas, caciques, oriole weavers and sun birds belong to this category.

Nesting Behaviour

The avian fraternity, diversity with regard to ecology and behaviour has been documented in several species. Nesting behaviour among others is an important feature in the class Aves. Amidst different taxa, species exhibit significant variations in their nesting ecology, such as selection of the nesting habitat, materials used for building nest, construction patterns, shape and size, and the feature of the nest (Collias and Collias, 1984; Hansell, 2000; Raval, 2011). Birds build their nest in the form of receptacle that holds eggs and/or the nestlings along with incubating parent (Soler *et al*, 1998a). In accompaniment, the nest renders desired microclimate for the development of nestlings (Collias and Collias, 1984) and also provides insulation to the birds, eggs and the young ones from adverse environmental conditions (Kern, 1987). However, the cost associated with preparation of the nest in terms of expenditure of time, energy and the predation risk during nest construction is enormous (Lima, 1987). Many avian species build their nests with durable materials that may last for more than one breeding season (Cavitt *et al*, 1999). Such long-lasting nests facilitate birds to re-use their nest which endows individuals with improved breeding success (Gauthier and Thomas, 1994), enhanced mating opportunities (Ueda, 1989) and increased foraging opporunity (Gauthier and Thomas, 1994). The reason behind such enhanced activities is the time and energy saved in re-use of old nests (Pearson, 1974; Ueda, 1989; Gauthier and Thomas, 1994). The pattern and the construction of a nest signify parental skilfulness, inherent skill, experience, physical strength of mates and ability to protect nest from the predators (Borgia, 1987; Zahavi, 1987; Soler, 1998b). Hence, nest construction behaviour of a bird provides clues about the mate quality, evolutionary lineage, energy cost, demographic trends and the life history traits of the species.

The main function of the nest in birds is to facilitate breeding but in some species, it can also be utilized for roosting during the non-breeding season (Hansell and Deeming, 2002). The nest holds eggs together and prevents individual eggs from rolling down (Heenan and Seymour, 2011) which might also minimize the risk of unequal incubation of the eggs (Tulp *et al*, 2012). A nest can also afford to provide protection from predators (Moller, 1987; Sanchez-Lafuente *et a*l, 1998). A concealed nest screened with dense vegetation also diminishes predation risk irrespective of whether the bird is on-bout or off-bout (Tulp *et al*, 2012). A properly lined nest prevents loss of heat by the eggs and also facilitates parent birds to maintain humidity in the nest (Hunsell, 2000; Ar and Sidis, 2002). Its significance in conservation of heat becomes apparent in cold environments with substantial offering to the parents, eggs and juveniles (Szentirmai *et al*, 2005). The insulation property of the nest also benefits the incubating birds in conserving energy expenditure (Buttemer *et al*, 1987). Nest presents a researcher with adequate evidence of phenotype of bird by providing detailed account of the behaviour embodied in construction of nest (Hansell, 1984; Collias, 1986). The detailed study of quantifiable elements used in the nest construction, its placing and ontogeny assist in comparative analysis among different species of birds (Zyskowski and Prum, 1999).

The birds through construction of nests do not only provide the protection and thermally uniform microclimate to the hatchlings, but they also optimize energy expenditure on nest construction (Collias and Collias, 1984; Hansell, 2000; Stanley, 2002). The systematic study of the nesting sites, nest construction behaviour and predation risk, all aid in tracing the phylogenetic history and ecology of species and their influence on life history traits (Collias, 1986; Martin, 1988, 1993; Martin and Li, 1992). Information on life history traits together with nesting and breeding success can help in assessing the abundance and vulnerability of species to populations and the inclination of the demographic trend before actual decline in the population density occurs (Primm *et al*, 1988; Pienkowski, 1991; Temple and Wiens, 1989).

Most of the avian species build nest every year, although, some of them may repair/re-construct their old nests by using easily available materials like hair, feathers and leaves to impart thermal insulation and anti-parasitic character (Clark and Masson, 1985; Hilton *et al*, 2004). The size and characteristics of nest reflects serious and sincere involvement of mates in the process of making nests that eventually add to the success story of reproduction (Moreno *et al*, 1994; Soler *et al*, 1998a, 1998b, Polo and Veiga, 2006). In most species, females take pro-active initiatives towards nest construction with modest help by the males. However, in few species of polygynous birds, male constructs most of the nest (Campbell and Lack, 1985; Metz *et al*, 2007). Nest construction behaviour of a bird species could be understood by exploring or examining (i) the perfectly completed nests (Walsh *et al*, 2010), (ii) the variation in structural complexity of fully constructed nest (Crook, 1964), (iii) the nest construction materials (Collias and Collias, 1984) and (iv) the construction behaviour of an individual (Metz *et al*, 2007). The duration for nest construction also varies considerably among species and the habitat, *for example*, weaver birds complete their nest in 18 days (Asokan *et al*, 2008), pied bush chat, 4-14 days (Sethi *et al*, 2010), swallows, 8-18 days (Lind, 1960), bank swallows, 1-16 days (Asbirk, 1976), and barn swallows construct cup type nest in about 6.4 days (Samuel, 1971).

Nest Protection

The most vital step towards continuation of the progeny is likely to prompt seasonal breeders to exercise maximum care in the process of raising chicks. Many species, despite placing their nests camouflaged with surroundings, establish territory and guard their nest and chicks to ensure safer fledging. Birds like red-vented bulbuls, stonechats, greater rhea, songbirds, zebra finches, humming birds and nuthatches guard their nest and the young ones (Goymann and Landys, 2011; Marasco *et al*, 2011; González-Gómez *et al*, 2014; Valdez *et al*, 2014; Prior *et al*, 2016; Pikus *et al*, 2017; Thibault *et al*, 2019; Randler and Randler, 2020). Fluctuations in the circulatory concentrations of sex steroids like progesterone, testosterone, dihydroepiandrosterone and their physiologically active metabolites have differently been involved in inducing territorial integrity of birds (Crews, 1984; Wingfield and Soma, 2002; Balthazart *et al*, 2004; Canoine *et al*, 2006; Pradhan *et al,* 2010; Marasco *et al*, 2011; Rosvall, *et al,* 2012; Tetel and Acharya, 2013; Wingfield *et al*, 2018; Watts, 2020). Progressive increases in the circulatory levels of sex steroids have been shown to induce culmination of mating behaviour in aves (Wingfield and Soma, 2002; Balthazart *et al*, 2004; Pradhan *et al*, 2010; Roswall *et al,* 2012; Tetel and Acharya, 2013; Wingfield *et al*, 2018; Watts, 2020).

Daylengths and Breeding Cycles

Baker (1938) based on extensive survey on passerine birds suggested that daylength may serve as the proximate factor in the boreal and temperate regions. In these regions, breeding is principally restricted to spring and summer months when increasing ambient temperature induces growth of vegetation and as a consequence, an increase in invertebrate and vertebrate food resources occur. In most of such species, breeding ends abruptly in the mid or late summer owing to cessation of stimulatory impact of long daylengths on the hypothalamo-hypophyseal-gonadal axis (photorefractoriness) and thus allowing the youngs to grow to sufficient size to survive seasonal scarcity of food or to be able to migrate (Turek and Campbell, 1979; Follett and Robinson, 1980; Sharp, 1984a,b; Wingfield *et al*, 1992). Between 30^{o}N and 30^{o}S, seasonal breeding is common but is not restricted to the months of the year corresponding to the spring and summer months of the temperate-zones (Sharp, 1984a,b; Dawson and Sharp, 2007; Dawson, 2008, Dawson, 2015). Moving towards the equator within subtropical regions breeding becomes less dependent on the seasonal changes in temperature and more dependent on rainfall (Immelman, 1971; Thapliyal, 1978, 1981; Chandola *et al*, 1983; Thapliyal and Gupta, 1979). In the equatorial region, intensity of isolation and/or precipitation appears to be the most significant signal in timing reproduction. Studies of Thompson (1950), Skutch (1950), Keast and Marshall (1954) have in general, confirmed Baker’s generalization. But some workers *viz*. Brown and Rollo (1940), Rollo and Domm (1943) and Wolfson (1959), on the basis of experiments made by them suggested that seasonal fluctuation in daylengths could be a regulatory factor not only in temperate-zones but even in the tropics. Experimental evidence gathered during the past few decades on birds from different latitudes also favour such an assumption. There are many species occupying equatorial and sub-tropical regions which have been found nesting during spring and/or summer months and their breeding is terminated by the intrusion of absolute photorefractoriness. In Japanese quail, *Coturnix coturnix japonica*, gonads of birds develop under long/longer daylengths but regress when the prevailing daylength is decreased even though light durations still being more than which had resulted their recrudescence earlier in the season (Robinson and Follett, 1982; Follett and Pearce-Kelly, 1990). Such birds are said to be relatively refractory and their gonads redevelop following transfer to long/longer daylengths (Nicholls *et al*, 1988; Dawson, 2015; Nakane and Yoshimura, 2019). These species differ only with regard to critical threshold of stimulatory daylengths which induce development and maturation of the reproductive system, but breeding ends under daylengths longer than which had initially induced development of the gonads. Photosensitivity in these birds is regained only when birds pass through winter like short daylengths (See reviews: Farner and Follett, 1966; Farner and Lewis, 1971; Turek and Campbell, 1979; Follett and Robinson, 1980; Thapliyal, 1981; Sharp, 1984a,b; Nicholls *et al*, 1988; Dawson, 2015; Kang, 2021). In some resident birds like wood pigeon, *Columba palumbus* (Lofts *et al*, 1967a), red-cross bill, *Loxia curvirostra* (MacDougall-Shackleton *et al*, 2006), rufous collared sparrow, *Zonotrichia capensis hypoleuca* (Lewis *et al*, 1974) and Indian weaver birds, *Ploceus philippinus* (Thapliyal and Saxena, 1964; Singh and Chandola, 1982; Chandola-Saklani *et al*, 1990) daylengths act as a driver and breeding terminates following decrease in daylengths during autumn and winter months (Lofts and Murton, 1968; Thapliyal and Gupta, 1989; Nicholls *et al*, 1988; Dawson, 2015). Such species, unlike those placed in the first category do not develop refractoriness to photostimulation and long daylengths irrespective of months or seasons, stimulate gonadotrophic functions. Third category includes birds which do not directly respond to photostimulation but long/longer days do impart crucial role in phasing some form of autonomous reproductive activity (Thapliyal, 1981; Chandola *et al*, 1983; Sharp, 1984a,b; Thapliyal and Gupta, 1989; Dawson, 2015). This assumption has most successfully been tested in a sub-tropical species like spotted munia, *Lonchura punctulata,* a monsoon breeder, in which autonomous testicular growth could be advanced by a few weeks in birds transferred from increasing to decreasing daylengths or the reverse photo-cycle (Chandola *et al*, 1983).

In tropical and equatorial regions seasonal fluctuations in daylengths are meagre or negligible and some birds have been noticed to become non-seasonal breeders (Immelman, 1971), but breeding in many is seasonal, depending primarily on the pattern of rainfall. For example, in the tropical rainforests of Sarawak, seasonal breeding coincides with flourishing of insects during the early part of the year (Fogden, 1972). This increase is induced by the new growth of vegetation which follow northeast monsoon between December and February. The breeding season of passerine birds in Sarawak is thus less sharply defined than in temperate-zone. Similar well defined breeding seasons have been reported for many bird species living in tropical forests in other parts of the world (Baker *et al*, 1940; Moreau, 1950; Snow and Snow, 1964). Seasonal breeding also depends on rainfall in other habitats such as the Savana grassland of Africa. For example, the red-billed quelea, *Quelea quelea* breeds only during the rainy season when there is an increase in the insect food supply (Jones and Ward, 1976). Birds migrate following rains and as they pass across the continent, they stop to breed when the opportunity occurs.

An underlying seasonality often occurs in species in which breeding appears to be erratic. For example, in the semi-arid inland region of south-western Australia, there is much year to year variation in the timing of breeding in different species. However, most birds tend to breed in the spring months when temperatures are rising after a heavy rainfall (Davies, 1979). In general, breeding rarely occurs in mid-summer when temperatures are high and food resources become scarce. The low temperatures of winter tend to inhibit breeding in many species, although in ground feeding insectivorous birds such as the banded plover, *Vanellus tricolor,* dotterel, *Peltohyas australis*, and the white-browed babbler, *Pomatostomus superciliosus,* winter breeding is common. Of the species inhabiting south-western Australia, for example, zebra finch, *Poephila guttata,* appears to breed in most months of the year. However, in this species there is an underlying pattern of seasonal breeding as the birds breed after rainfall in the spring when temperatures are rising. Rainfall in the autumn is less likely to induce breeding in this species (Davies, 1977). These observations support the view that seasonal breeding is common at all latitudes and in many habitats and the nestlings hatched early in the breeding season are more likely to survive than those hatched late (Perrins, 1970). It is probably of adaptive value for any seasonal breeder to predict and prepare for a breeding season. The question whether a bird can predict a time favourable for breeding can sometimes be difficult to answer because of the role of the maternal food supply as a source of proximate information (Sharp, 1984a).

Body Mass Cycles

Likewise gonads, body mass of birds has been shown to vary during the breeding season and the change becomes more pronounced in migratory birds (Thapliyal, 1969, 1981; Immelman, 1971;

Clark, Jr, 1979; Araujo *et al*, 2019) as compared to resident passerine and non-passerine species. For example, in non-migratoty pheasants, *Phasianus colchicus* (Kirkpatric, 1944; Kobat *et al*, 1956; Clarence *et al*, 1962), black-headed munia, *Munia malacca malacca* (Thapliyal and Pandha, 1967a), lal munia, *Estrilda amandava* (Thapliyal and Pandha 1967c; Thapliyal and Gupta, 1984; Thapliyal *et al*, 1991; Lal and Thapliyal, 1995), chest-nut bellied munia, *Munia atricapilla* (Thapliyal and Garg, 1967; Thapliyal and Bageshwar, 1970), spotted munia, *Uroloncha punctulata* (Thapliyal, 1968; Vidyarani and Lal, 2007; Lal *et al*, 2012), white-winged black bird, *Agelains phoeniceus* (Robinson and Rogers, 1979), rook, *Corvus frugilegus* (Lincoln *et al*, 1980), Indian spotted doves, *Streptopelia chinensis* (Malik and Lal, 1998) and tree sparrows, *Passer montanus* (Lal *et al*, 2007), birds tend to economize their energy resources to meet the calorie requirements during the process of raising the progeny. In sedentary spotted munia, castration or exogenous treatments of gonadal steroids has been reported have no obvious influence on body weight of birds (Thapliyal, 1968). However, influence of sex steroids on body weight has been reported to differ depending on the doses administered and the physiological/sexual stage of birds at the time of administration (Gupta and Thapliyal, 1984). In some findings sex steroids have been shown to induce pre-migratory lipid storage in the adipose tissues of birds (Weise, 1967; Tewary and Kumar, 1981; Thapliyal *et al*, 1983) and the effects seem to appear through augmented release of Prolactin from the adenohypophysis (Yokoyama, 1976, 1977). Prolactin has been shown to influence the gonads and body weight of both, migratory and resident birds (Chandola and Pavgi, 1979; Thapliyal and Lal, 1984a; Seiler *et al*, 1992; Thapliyal, 1997). Quality and/or quantity food intake and diurnal feeding trends have variously been reported to influence body weight of avian species (Farner, 1975; Chandola *et al*, 1980; Thapliyal and Gupta, 1989; Thapliyal, 1981; 1997).

Thyroid Hormones and Breeding Cycles

Environmental variables trigger the cascade of neuroendocrine events which lead to the coordination of metabolic preparations and the maturation of reproductive system. Thyroid hormones are known to establish linkages with neuroendocrine-gonadal and neuroendocrine-metabolic axes during the course of sexual and body mass cycles of birds and mammals (Thapliyal, 1981; Nicholls *et al*, 1988). During the past few decades several reviews have been made on this aspect of avian endocrinology (Thapliyal, 1969, 1978, 1981; Assenmacher, 1973; Assenmacher and Jallageas, 1978, 1980; Nicholls *et al*, 1988; Dawson *et al*, 2001; Yashimura, 2013). Existing literature describe profound inter and intra-species variations in thyroidal regulation of neuroendocrine-gonadal axis of birds. In many species, active thyroid glands seem to be essential for the development and maturation of gonads. In such avian forms, extirpation of thyroid glands precipitates gonadal involution and exogenous administration of thyroid hormones successfully reverses this effect (Benoit and Aron, 1934; Benoit, 1936; Blivaiss and Domm, 1942; Caridroit, 1943; Payne, 1944; Shaffner and Andrews, 1948; Kumaran and Turner, 1949; Vyas and Ramaswami, 1965; Thapliyal and Garg, 1969; Jallageas and Assenmacher, 1974; Lal and Thapliyal, 1982a; Lal and Pathak, 1987; Lal, 1988; Singh *et al*, 1992, 1993; Devi and Lal, 1992,1994; Malik and Lal, 1998; Dawson, 1998; Wilson and Reinert, 1995, 1996, 2000; Yoshimura, 2013; Nabi *et al*, 2020). By sharp contrast, in certain species active thyroid glands are essential for preventing unseasonal development of gonads (Thapliyal, 1969; 1978, 1981; Nicholls *et al*, 1988; Yoshimura, 2013). In such birds, thyroidectomy results precocious development of gonads which develop fully and then remain active for extended periods or the post-nuptial regression is eliminated (Woitkewitsch, 1940; Thapliyal and Garg, 1967; Thapliyal and Bageshwar, 1970; Thapliyal and Chandola, 1972; Thapliyal 1981; Wieselthier and Van Tienhoven, 1972; Chaturvedi and Thapliyal, 1983; Goldsmith and Nicholls, 1984b; Follett and Nicholls, 1984; Nicholls *et al*, 1988; Dawson, 1993). In all such species, treatment with exogenous thyroid hormones precipitates premature gonadal regression (Thapliyal, 1969, 1978, 1981; Assenmacher and Jallageas, 1978, 1980; Silverin, 1980; Nicholls *et al*, 1988).

In some species, effects of thyroidectomy and/or L-thyroxine treatments have been examined during different stages of the gonad development cycles. In house sparrow, *Passer domesticus* (Lal and Thapliyal, 1982b, Dawson 1993), tree sparrow, *Passer montanus* (Lal and Pathak, 1987), red-headed bunting, *Emberiza bruniceps* (Lal, 1988) and black-headed bunting, *Emberiza melanocephala* (Devi and Lal, 1992, 1994), thyroidectomy prevents/suppresses testicular growth, results premature involution of fully developed testes but has no effects on gonads when performed during the quiescent and/or regression phases of the gonadal cycle. In common myna, *Acridothres tristis*, duck, *Anas platyrhynchos* and teal, *Anas crecca*, thyroidectomy during progressive phase inhibits gonadal growth but extends the active phase of testes when performed during breeding phase (Assenmacher and Jallageas, 1978, 1980; Chaturvedi and Thapliyal, 1983). Moderate doses of L-thyroxine administered during progressive phase promoted testicular growth but led to regression of gonads when employed during peak phase in house sparrow (Vaughan, 1954). On the contrary, in common myna, L-thyroxine suppressed testicular growth during progressive phase but extended the active phase of gonads for at least two months when employed in birds with fully developed gonads (Chaturvedi and Thapliyal, 1983).

Thyroid hormones have also been implicated in the termination of avian breeding cycles and the influence of thyroid ablation and/or challenges with exogenous thyroxine (L-T_4) have produced varying effects amongst species. Regressing gonads of common myna, *Acridotheres tristis*, house sparrow, *Passer domesticus*, tree sparrows, *Passer montanus*, *Spizella arborea*, red-headed bunting, *Emberiza bruniceps*, black-headed bunting, *Emberiza melanocephala* and spotted dove, *Streptopelia chinensis* (Chaturvedi and Thapliyal, 1980a; Lal and Thapliyal, 1982b; Lal and Pathak, 1987; Lal, 1988; Wilson and Reinert, 1996, 2000; Devi and Lal, 1992, 1994; Malik and Lal, 1998), thyroid hormones fail to influence gonadal regression. However, in duck, *Anas platyrynchos* and teal, *Anas crecca* (Assenmacher and Jallageas, 1978, 1980), white crowned sparrow, *Zonotrichia leucophrys gambelii* (Smith, 1978, 1980); Japanese Quail, *Coturnix coturnix japonica* (Follett and Nicholls, 1984; Chaturvedi and Meier, 1989) and European starlings, *Sturnus vulgaris* (Nicholls *et al*, 1988), thyroid hormones seem to be critically involved in the termination of seasonal breeding by the development of photorefractoriness (Assenmacher and Jallageas, 1978, 1980).

Thyroid Hormones and Body Mass Cycles

Thyroid hormones have been shown to influence seasonal changes in body weight of birds (Thapliyal and Bageshwar, 1970; Thapliyal, 1981, 1992, 1997; Lal and Thapliyal, 1985a; Thapliyal *et al*, 1991). Both, long and short-term experiments made on different species of birds denote that absence of thyroid hormones increase body weight of spotted munia, *Lonchura punctulata*, lal munia, *Estrilda amandava*, chestnut-bellied munia, *Munia atricapoilla*, weaver bird, *Ploceus philippinus*, rain quail, *Coturnix coturnix coromandelica* and European starling, *Sturnus vulgaris* (Thapliyal and Garg, 1967; 1969; Thapliyal, 1969; Thapliyal and Bageshwar, 1970; Weiselthier and Van Tienhoven, 1972; Saxena and Saxena, 1975; Thapliyal, 1992), decrease body mass of Indian starlings, *Sturnus roseus*, common grey quail, *Coturnix coturnix*, Indian Spotted dove, *Streptopelia chinensis* (Shah et *al*, 1977; Saxena and Saxena; 1979; Malik and al,

1998) or have no effects on body weight of common myna, *Acridotheres tristis*, red-vented bulbul, *Molpastes cafer*, house sparrow, *Passer domesticus* and tree sparrow, *Passer montanus* (Chaturvedi and Thapliyal, 1979, Lal and Thapliyal, 1982a,b; Lal and Pathak, 1987). In red-headed, *Emberiza bruniceps* and black-headed *Emberiza melanocephala* buntings, thyroidectomy decreases body weight during progressive and peak phases but has no effects when performed during quiescent or regression phases of the body weight cycle (Lal and Thapliyal, 1985a; Lal, 1988; Devi and Lal, 1992, 1994). Thus, every species appears to be unique in the way they utilize thyroid hormones to tackle their metabolic mechanisms during the course of gonadal maturation and display of associated behavioural attributes. The mechanism of thyroid hormone regulation of neuroendocrine-gonadal axis of birds has been elaborated in Japanese quail, *Coturnix coturnix japonica* and the findings appear to have great analogy with mammals (Yashimura, 2013). Stimulatory photoperiods have been reported to enhance the synthesis of THRα, THRβ, and THRϰ receptors *vis a vis* increased expression of Dio2 gene which up regulates conversion of T_4 (thyroxine) into active T_3 (triiodothyronine) (Bernal, 2002) in the ependymal cells bordering ventrolateral walls of third ventricle within mediobasal-hypothalamus (MBH) (Yashimura *et al*, 2003). This cascade has been observed to operate at low ebb under short photoperiodic regime to uphold the regulation of Dio3 gene expression leading to conversion of T_4 into reverse -T_3 (inactive form of T_3) and metabolites, dominated by T2 (3’5’ diiodotyrosine) (Yasuo *et al*, 2005). Reciprocal role of Dio2 and Dio3 genes within mediobasalhypothalamus under stimulatory and inhibitory daylengths seems to be instrumental in the development of seasonality in photoperiodic species. It has been observed that under long daylengths local availability of T_3 within MBH rise up to 10 folds as compared to non-stimulatory daylengths whereas no obvious differences in plasma T_3 profiles were noticed (Yashimura *et al*, 2003). The functional significance of high levels of T_3 in MBH under long daylengths became obvious when infusion of T_3 into third ventricle of Japanese quail on non-stimulatory daylengths resulted testicular recrudescence in a dose dependent manner and this response vanished when Dio2 gene expression was masked with iopanoic acid (Yashimura *et al*, 2003). Dio2 and Dio3 gene expressions have been shown to have linkages with seasonal variations in natural lighting hours in species like tree sparrows, chicken, great tits and canaries (Watanabe *et al*, 2007, Ono *et al*, 2009, Perfito *et al*, 2012, Stevenson and Ball, 2012). Further, electron microscopy of MBH in Japanese quail exhibited striking structural dissimilarities at GnRH nurones and their glial endfeet in birds kept on stimulatory (LD) and inhibitory (SD) daylengths (Yamamura *et al*, 2004). These observations have great similarities with mammalian species which exhibit profound dissimilarities in morphological features of GnRH nerve endings and their glial endfeet (Jansen *et al*, 2003). In Japanese quail kept on long daylengths, GnRH nerve endings were found to be in contact with pericapillary space (Prevot *et al*, 1999) which was seen obstructed by thick glial endfeet in birds exposed to non-stimulatory photoperiods (Yamamura *et al*, 2006). This daylength dependent disparity in morphological features of GnRH neurones and glial cells were found to be associated with the levels of T_3 in the ependymal cells bordering MBH as intracerebroventricular infusion of T_3 had resolved differences in anatomical features of nerve endings and the glial cells of birds kept on long and short daylengths. An obvious consensus seems to be evolving with central focus that seasonal plasticity in daylengths influence hypothalamic content of T_3 to effect temporal changes in anatomical structures of GnRH nerves and the glial cells with consequent modulation of GnRH release in birds and mammals. This concept however, fails to explain observation in European starlings, *Sturnus vulgaris* in which Dio2 gene expression is not correlated with seasonal diversity in testicular development and regression (Bentley *et al*, 2013). This only report may be crucial to prompt important query whether similar mechanisms may be operative in other avian species in which likewise European starlings reciprocal relationship exists between thyroid hormones and gonadal development? Obviously, this demands further insight into the concern and many more species need to be examined to help emerge an unequivocal concept. This creates scope for further evaluation of possible cross-talk between hypothalamo-hypophyseal-gonadal, hypothalamo-hypophyseal-thyroidal and hypothalamo-hypophyseal-metabolic axes of birds from different latitudes and varied eco-habitats.

From the foregoing literature, it may be construed that likewise other vertebrates, birds exhibit great adaptive radiations in their neuroendocrine mechanisms to enhance their survival fitness. Accordingly, species tend to adapt newer metabolic, physiologic and behavioural strategies to cope with habitat specific requirements. In the present study, this possibility was examined in red-vented bulbul, *Pycnonotus cafer stanfordi* to address following aspects of their reproductive physiology at (26°N).

1. Thorough assessment of seasonality in food intake, body mass, gonad size, activity and expression of associated reproductive behavioural attributes, parental care and feather renewal.

2. Evaluation of seasonal changes in the sensitivity of light responding mechanisms and

3. Possible involvement of light and thyroid hormones in the regulation of gonadal and body mass cycles of red-vented bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami, Zunheboto District, Nagaland State.

CHAPTER – II

Seasonal Changes in Feeding Ecology, Body Mass, Gonad Size and Activity, Moult and Reproductive Behaviour of Red-Vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami, Zunheboto District, Nagaland State

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INTRODUCTION

Literature on phenology of gonads, body mass and plumage renewal process of birds have been extended in many species (See reviews: Farner and Follett, 1966; Lofts and Murton, 1968; Immelman, 1971; Murton and Westwood, 1977; Thapliyal, 1978, 1981; Sharp, 1984a; Thapliyal and Gupta, 1989; Chandola-Saklani *et al*, 1991; Wingfield and Farner, 1993; Dawson, 2008; Xiao *et al,* 2017). However, bulk of literature pertains either to the domestic species or to the temperate-zone migratory birds and our knowledge on breeding biology of birds from tropical and sub-tropical parts of the Indian sub-continent is limited (see reviews: Thapliyal, 1969, 1978, 1981; Thapliyal and Gupta, 1989). Out of nearly 10,000 extant species of birds in the world today, India alone hosts about 1200 species (Ali and Ripley, 1996). During the past few decades, gonad development cycles of approximately four dozen bird species have been described and about a dozen of them have been studied experimentally (Thapliyal, 1954, 1968, 1969, 1978, 1981, 1992, 1993, 1997; Chandola and Thapliyal, 1978; Chandola-Saklani *et al*, 1988a, 1991; Thapliyal and Gupta, 1984, 1989; Malik and Lal, 1998; Dixit and Singh, 2011; Lal *et al*, 2012; Dixit and Byrsat, 2018). However, in most of these studies, findings on seasonal variations in body mass and size and activity of gonads pertain to the laboratory investigations made at Varanasi (Lat. 25°18'N; Long. 83°01'E) by Thapliyal and co-workers and birds from other parts of the Indian dominion have received relatively less attention (Gupta and Maiti, 1987, 1988; Salaja *et al.*, 1988; Narasimhacharya *et al.*, 1988; Chandola-Saklani *et al*, 1990; Dixit and Singh, 2011; Lal *et al*, 2012). Further, less is known about feather renewal process in Indian birds (Dixit and Singh, 2011). In the present study, efforts have been made to examine the seasonal changes in body mass, gonad size and activity, breeding conduct and the feather renewal in the captive and free living populations of red-vented bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami (Lat. 26^{o}13’ N, Long. 94^{o}28’ E), Zunheboto district, Nagaland state.

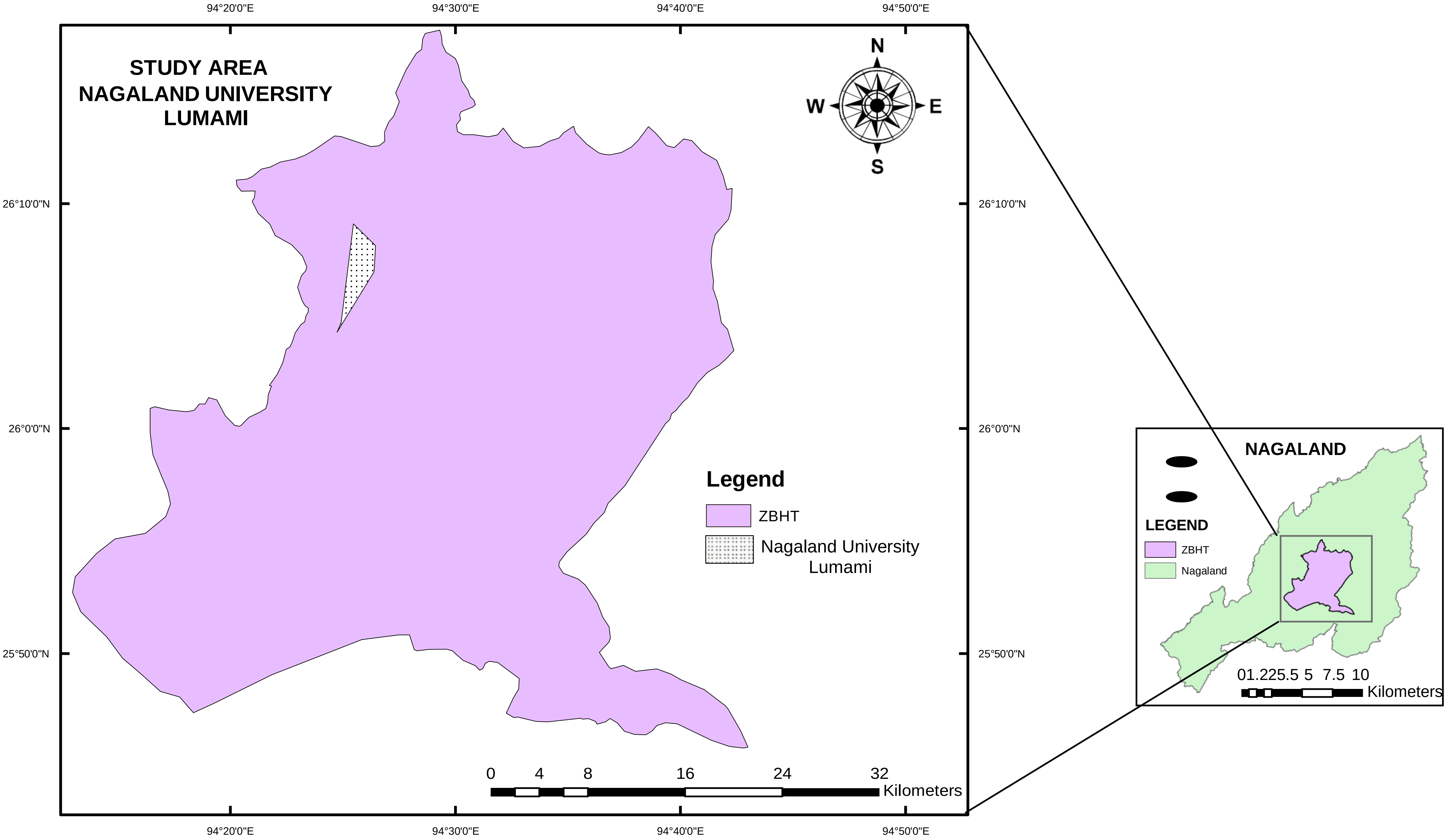
MATERIALS AND METHODS

Red-vented Bulbul (*Pycnonotus cafer stanfordi*) is a non-migratory finch which belongs to the family *Pycnonotidae*. This avian family incorporates 138 species of bulbuls detailed under 27 Genera. The Genus *Pycnonotus* comprises of 47 species (del Hoyo *et al*, 2005; Thibault *et al*, 2018a,b, 2019; Nowakowski and Dulisz, 2019), out of which 6 species: black-headed bulbul (*Pycnonotus atriceps atriceps,* Temminck,1822); black-crested yellow bulbul (*Pycnonotus melanicterus flaviventris* Tickell 1833); Assam red-whiskered bulbul *(Pycnonotus jocosus monticola,* McClelland, 1840); Burmese red-vented bulbul (*Pycnonotus cafer stanfordi,* Deignan 1949); Mishmi striated green bulbul (*Pycnonotus striatus arctus,* Ripley 1948); Blyth’s bulbul (*Pycnonotus flavescens flavescens,* Blyth 1845) are found in Nagaland (ZSI, 2006). The Species, *Pycnonotus cafer* has 8 sub-species, of which, *Pycnonotus cafer stanfordi* is found in most parts of the North-East Indian states: southern part of Assam, Arunachal Pradesh, Nagaland, Manipur, Meghalaya, Tripura, Bangladesh, northern Myanmar and south-western parts of the Chinese province between 23^{o}N-29^{o}N (ZSI, 2006).

STUDY SITE

Present study was undertaken at Lumami (26^{o}N) in Zunheboto district of Nagaland state (Map 1). The field study area was divided into 4 segments (A-D), each having 2-3 different observational spots around Nagaland university hqrs at Lumami. Segment A covered approximately 1.5 km area in the North, segment B ranged for about 2 km area in the South, Segment C covered 1.2 km area in the South-West of the university campus and Segment D included more than 2 km area bordering university campus on the Eastern side.

Starting from January up to December 2017, some freshly shot birds were purchased from the local hunters between 13-15th of every month. Body mass of individual bird was recorded, moult status was noted and then body cavity was opened to measure long and short axes of testes in male and diameter of largest ovarian follicle in female. Gonads and gonoducts were excised, weighed, cleaned and fixed in Bouin’s fluid for microscopic study. Guts were excised


STUDY AREA
NAGALAND UNIVERSITY
LUMAMI
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0
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24
32
Kilometers
94°20'0"E
94°30'0"E
94°40'0"E
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26°10'0"N
26°0'0"N
25°50'0"N
NAGALAND
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Kilometers

and fixed in 5% formalin solution for analysis of food contents. Further, during the first week of January 2017, some birds were caught using birds' glue and some purchased from the local bird catchers and acclimatized to laboratory conditions for at least a fortnight before use. Birds were laparotomized, 6 males and 6 females were segregated into pairs in wire-mesh cages (2.5' x 2' x 1.5' in size). Cages were placed in a room on the wooden rack fixed against a window facing north. The room received unrestricted natural light and air. Records on individual body mass, moult status, left testis size (male), and diameter of the largest follicle *in situ* (female), were maintained regularly on 15th of every month until December 2017.

Observations on primaries of wing feathers (PFs) and body feathers (BFs) were recorded at monthly intervals following the technique described by Dhont (1977). Scoring pattern for PFs were made in a scale of 0-5, detailed as: 0=worn or old feather in place; 1=missing feather (just dropped); 2=a new feather papilla grown up to one-third; 3=new feather grown up to two-third; 4=new feather grown above two-third but not yet complete and 5=fully grown new feather. For each PF a minimum score of 0 and maximum of 5 was assigned. As there are 10 primaries in each wing, the maximum score per wing could make up to 50 (5x10=50) and for each bird a maximum score of 100 (50x2=100) could be expected. Body moult assessment was made by dividing the whole body of the bird into 12 different regions as: 1=head; 2=neck; 3=shoulder; 4=back; 5=pelvis; 6=caudal; 7=throat; 8=chest; 9=abdomen; 10=flank; 11=shank; and 12=sub-caudal. Each region was assigned a score of either 0 (fully grown or old feather in place) or 01 (dropped but no new feather emerging) and hence BFs moult score ranged from 0-12 (Dixit and Singh, 2011).

Between January 2017 and August 2021, regular observations on food and feeding patterns and different aspects of behavioural attributes such as pair-bonding, courtship display, nest-building, territory establishment, copulation/mating, egg laying, clutch size, incubation, development of chicks and parental care were recorded in the breeding populations by directly attending to the nests and/or using binocular (Canon-60x60) depending upon the topography of land and the nest-location. Canon EOS 700D-DSLR camera was used to accomplish photographic details. Tissues were cleaned, weighed and then fixed in the Bouin's fluid for examining microscopic details. Electronic balance (Wensar Class-II) was used to record the weight of eggs, and large sized gonads with minimum accuracy of 100*mg*. Off-season gonads and gonoducts were weighed using electronic balance (*K*-Roy) with the accuracy of 1mg.

Left Testis and largest follicle sizes were measured following exploratory laparotomy under the influence of Nembutal (Sodium Pentobarbital, 20mg/kg body mass, Indian Schering Ltd.). Birds were anaesthetized and fixed on a wooden surgical board by the right side. Area between left thigh and lower ribs was deplumed and a horizontal slit was placed between last two ribs to gain access to the gonads. Ribs were parked with the help of home-made retractors and diameter of largest follicle or length and width of left testis *in situ*, were measured using fine divider with the accuracy at 0.5 mm. Follicles below 0.5 mm could not be measured with precision and hence assigned a generalized score of 0.25 mm for the sake of computational convenience. Ribs were tied together and the wound was sutured with the help of silk thread. Neosporin (Johnson and Johnson) dusting was made to prevent infection.

Testis volume was calculated by using Bissonnett's formula *i.e.* $4/3\pi ab^2$, where a being the half of the long-axis and b, the half of the short-axis of the left testis as described by Lal (1988). Diameter and depth of nests were recorded using a fine divider and plastic scale. Nest-basement area was calculated using the formula $4/3\ \pi\ a^2b$, where a represents the radius and b, the height of the nest as detailed by Moller *et al.* (2014).

Clutch size manipulations were made exchanging freshly deposited conspecific eggs in the nest. Food contents were assessed by dissecting out guts to separate gut contents of individual bird in separate watch glasses using a fine brush and then record their weight (wet-mass). Plant and animal contents of the food were further separated, weighed and then analysed using dissecting microscope. In the captivity, birds were provided with Sattoo-paste (a preparation from roasted-gram flour) flavoured with honey, insects and their larvae, and seasonally available fruits (banana, papaya, cherry, guava, mulberry *etc*) and water *ad libitum* and remained in good health throughout the period of observation.

Meteorological data is based on Agromet Advisory Bulletin for Zunheboto district. Daylength varied at a maximum of 13hrs, 30 min (21st June) and a minimum of 10hrs, 30min (22nd December). Maximum and minimum temperature during the study period were recorded at

31.3 ^{oC} (August) and 7.7^{oC} (December) respectively. Relative humidity was maximum at 84.2% in August and minimum at 52.2% in February 2017.

Data were analysed using one way independent and repeated measures of analysis of variance (ANOVA). Student's '*t*' test was used to compare individual means. Significance was determined at 95% confidence limit ($p<0.05$) (Snedecor, 1961).

RESULTS

Food and Feeding Ecology: Red-vented bulbuls consume plant and animal food resources with obvious preferences depending on the season and the availability of food resources in the habitat (Plate-V, 1-12). Present results show that plant ($F_{(11,36)}= 6.22$, $p<0.0001$) and animal ($F_{(11,36)}= 3.31$, $p<0.001$, ANOVA) contents in the food consumed by red-vented bulbuls across seasons differ significantly despite no appreciable change in the total gut-contents ($F_{(11,36)}= 1.76$). Plants and/or their products as food contents were maximum during February and minimum in July-August. Conversely, invertebrate contents were lowest during February and highest during August. From June to September periods, plant materials in the gut-contents were significantly low as compared to their values obtained during February. In sharp contrast, invertebrate food in the gut content was significantly high in August as compared to values obtained from January-May and November-December (Table 1, Fig. 1). A large variety of plants or their products, *for example,* nectar from Indian coral trees, *Erythrina variegata* and *Erythrina indica*, banana, *Musa acuminata*, jamun, *Syzygium cumini*, guava, *Psidium quajava*, papaya, *Carica papaya*, lichi, *Lichi chinensis*, banyan figs, *Ficus benghalensis*, mulbery, *Morus nigra*, goose berry, *Phyllanthus emblica*, Cornelian cherry, *Cornus mas*, Australian cherry, *Syzygium paniculatum*, wild cherry, *Prunus aviam*, plums, *Prunus domestica,* blackshade berry, *Solanum nigrum,* ivy gourd, *Coccinia grandis,* velvetleaf fruits, *Abutilon theophrasti*, lantana, *Lantana camara* nectar, fruits and seeds, bottle brush flower, *Callistemon spps* nectar were consumed to support nutritional requirements of red-vented bulbuls. In addition, invertebrates, such as mosquitoes: *Aedes albopictus*, *Anopheles gambiae* cockroaches: *Periplanatus americana*, *Blattella germanica*, *Blattella orientalis*, ants: *Componotus spps*, *Lasius niger*, *Solenopsis spps,* grass hoppers: *Schistocerca americana*, *Phyllochoreia ramakrishnai*, *Oseudoproscopia scabra*, *Schistocera gregaria*, cicadas: cicacoidea, bush crickets: Tettigoniidae, thrips: winged and wingless forms, aphids: winged and wingless forms, termites:

1 2 3

4 5 6

7 8 9

10 11 12

Plate V. Photographs showing feeding ecology of Red-vented bulbul

Collection of plants and insect food items:
1.Ripe Banana;2-3. Ripe Papaya; 4-5 Ripe Guava; 6. Tomato; 7. Nectar from Indian Coral tree flower; 8. Holding Australian cherry; 9. Holding grasshopper; 10. Holding winged termite; 11-12. Group feeding on dropped wild cherry fruits.

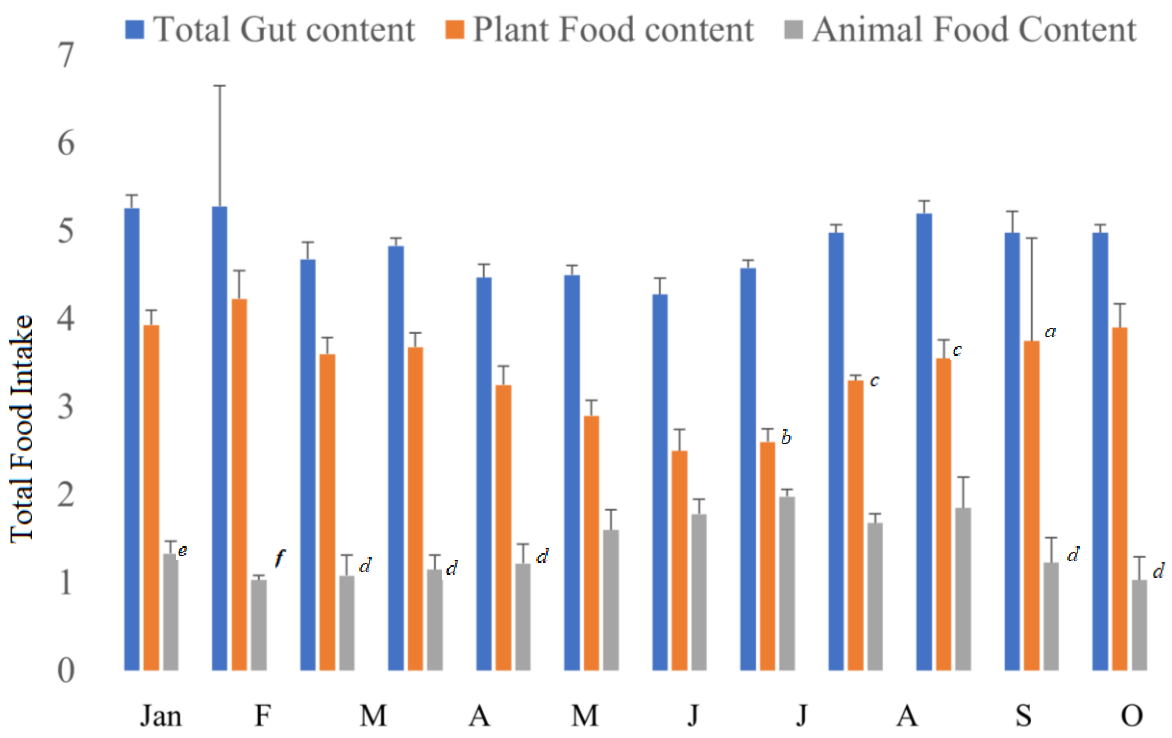

Fig. 1: Seasonal changes in Animal and Plant food consumption in wild Red-vented bulbul,*Pycnonotus cafer stanfordi*. Values are expressed as Mean±*SE*; n=4 in each group.Total gut contents across months, F($_{11,36}$) =1.76 (ANOVA). Plant food in gut-content across months, F($_{11,36}$) = 6.22, $p<0.0001$ (ANOVA), Animal food in gut-content across months, F($_{11,36}$) = 3.31, $p<0.001$(ANOVA); *a,b,c* differ from the value of February at $p<0.05$, <0.01 and <0.001 level respectively (Student's *t* test); *d,e,f* differ from the August value at $p<0.05$, <0.01 and <0.001 level respectively (Student's *t* test).

winged and wingless forms, *Isoptera spps, etc* were found to add to the quality food resources across months/seasons. Seasonal diversity in the food consumed became noticeable from November-February with plant contents making the major part of food consumed (~80%). Plant food resources like guava, flower buds, nectar, Mexican sunflower seeds and invertebrate food like ants, honey bees and flies made the preferred food consumed during this period. During March-May period, major part of the gut-contents (<70%) were plant materials like nectar, flower buds, leaves and petals, berries, cherry fruits, and animal contents like ants, grass hoppers, thrips, aphids and termites. However, from June to October period, animal food resources such as spiders, gross hoppers, ants, winged-termites had significantly increased (~35%), and plant food contents (nectar, small flower buds, mixed-plant seeds, banana, papaya, guava, plums, black nightshade and velvetleaf fruits) had significantly decreased in the gut-content as compared to their values obtained from November-June period (Table 1, Fig. 1).

Seasonal alterations in Gonad Size and Gonads, Gonoducts Mass: Gonads of both the sexes of bulbuls experienced significant seasonal variations in their size: male ($F_{(11,60)}$= 32.92, p<0.0001 and female: $F_{(11,60)}$=118, p<0.0001). In captive males, testes began developing during February-March, grew rapidly through March and April, attained peak (125.79±13.31mm^3) in May, decreased significantly (p<0.05) during May-June and thereafter rapid decline ensued to make them reduce to smaller size in September. Within group testicular size of birds in May measured significantly more as compared to values in other months (Table 2, Fig. 2A). Follicular cycle closely followed testicular cycle with seasonal peak appearing in May (2.63±0.11mm) and June (2.70±0.14mm). Within group follicular diameters of birds during May and June measured significantly more compared with values of other months (Table3, Fig. 3A). The pattern of seasonal changes in gonads of captive and free living birds were temporally similar, but peak testicular size (216.26±11.12mm^3) and follicular diameter (3.67±0.12mm) in May were significantly more in free living birds as compared to peak testicular (125.79±13.31mm^3) and follicular (2.50±0.11mm) sizes of captive bulbuls (Tables 2-5, Figs. 2-5A). Mean follicular sizes in captive birds from September-November were significantly (p<0.001) less as compared to values of free living females (Tables 3,5; Fig. 3A,5A).

Paired testis mass of free living birds differed significantly across the season ($F_{(11,60)}$=71.56, p<0.0001) and closely followed testicular cycle with an annual maximum of 224.95±31.45mg

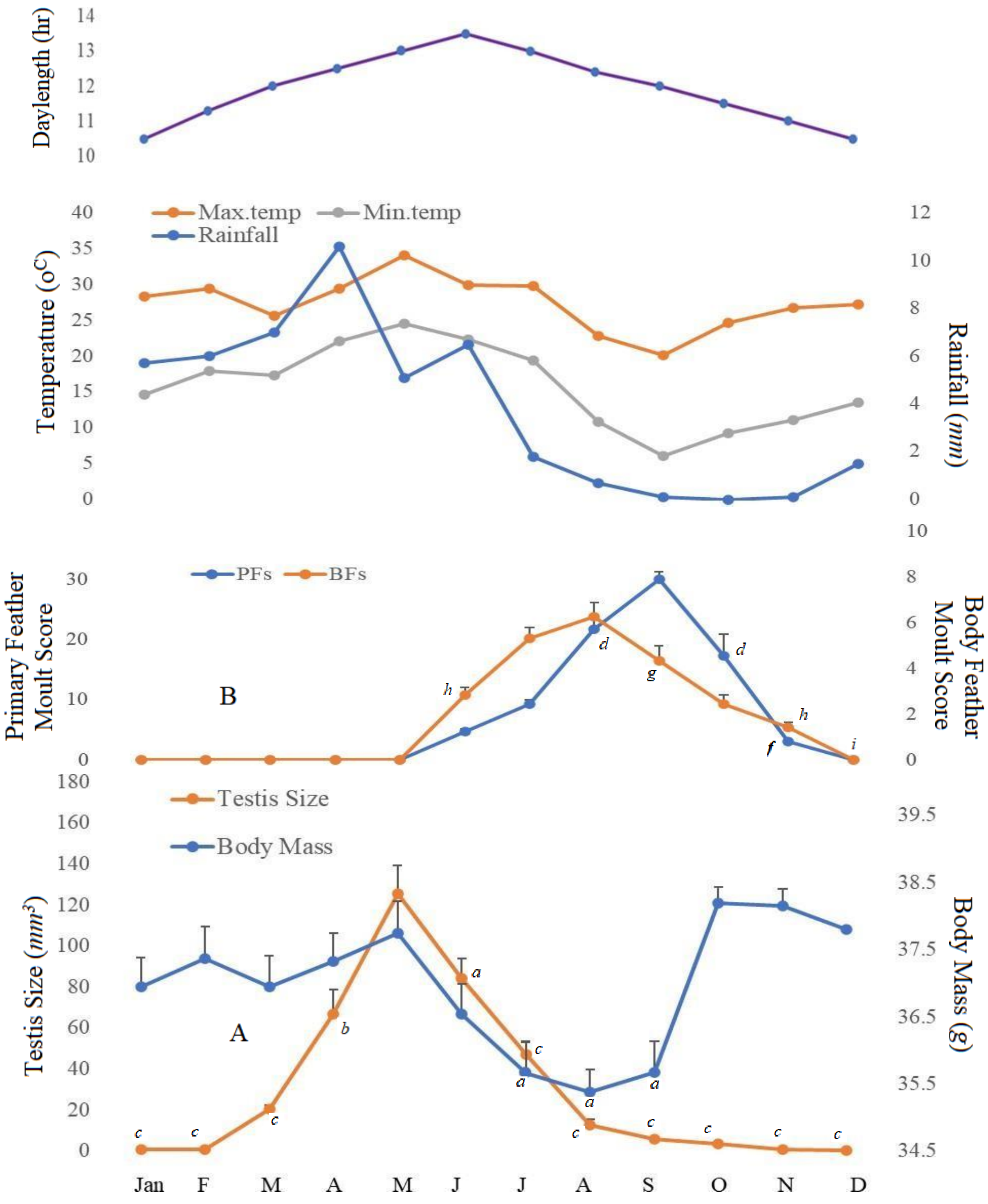

Fig.2: Seasonal changes in Testicular Size, Body Mass and Moult Score of Captive Red-vented bulbul, *Pycnonotus cafer stanfordi* at Lumami (26^{o}N).Values are Mean± *SE*, n=6; Body Mass:F($_{11,60}$)=5.44, p<0.001; Testicular Volume: F($_{11,60}$)=32.92, p<0.0001; Primary Feather Moult Score: F($_{5,30}$)=36,26, p<0.0001; Body Feather Moult Score: F($_{5,30}$)=12.07, p<0.0001 (One way ANOVA). *a,b,c* differ from May value at p<0.05, p<0.01 and p<0.001 respectively. *d,f* differ from the value of PF Score in September at p<0.05 and <0.001 respectively. *g,h,i* differ from the value of BF Score in August at p<0.05, p<0.01and <0.001 respectively (Student's *'t'* test).

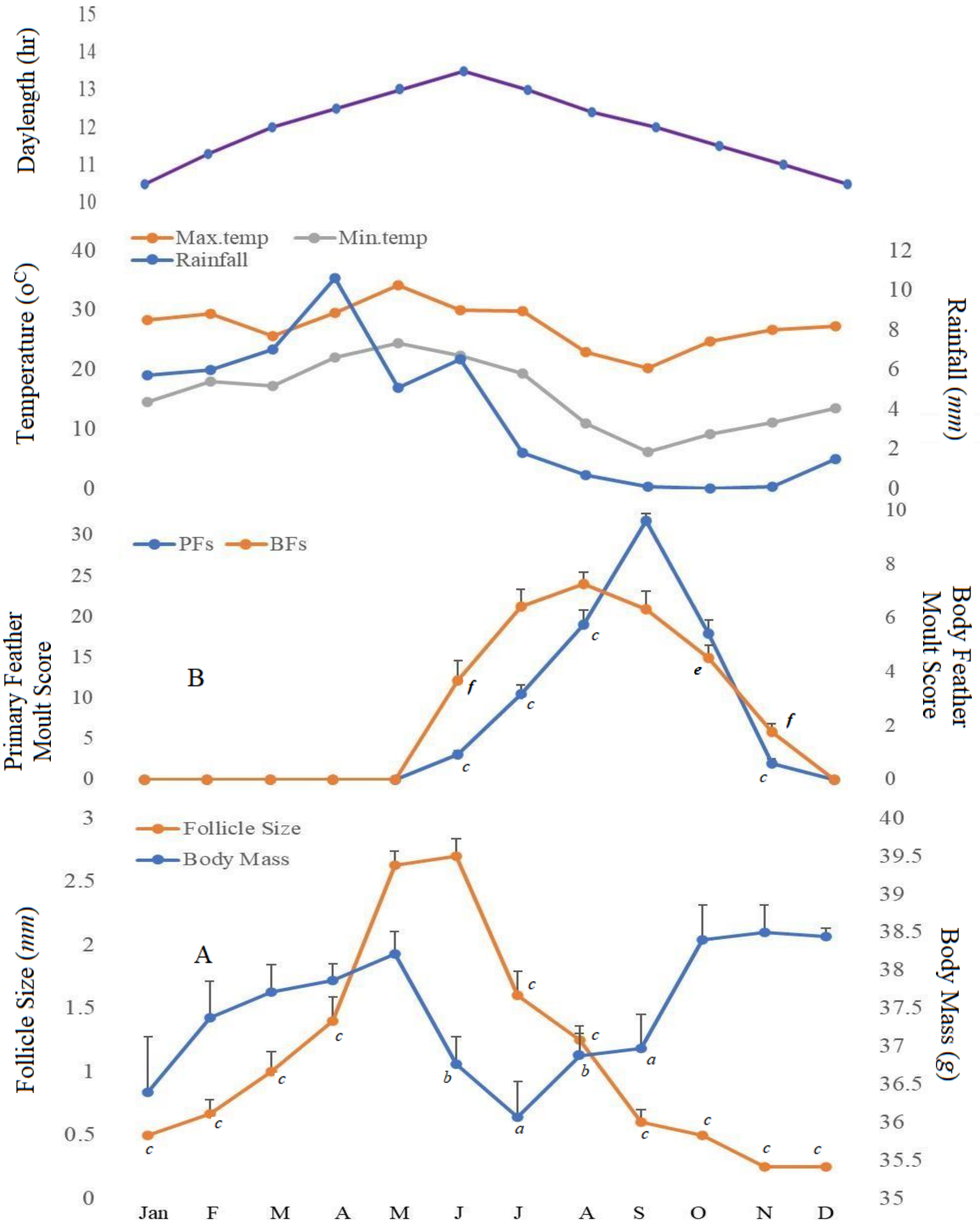

Fig.3: Seasonal changes in Follicular Size and Body Mass of Captive Red-vented bulbul, *Pycnonotus cafer stanfordi* at Lumami (26^{o}N). Values are Mean ± *SE*, n=6; Body Mass: $F(_{11,60})=3.04$, $p<0.005$; Follicular Size: $F(_{11,60})=118$, $p<0.0001$; Primary feather Moult Score: $F(_{5,30})=87.77$, $p<0.0001$; Body Feather Moult Score: $F(_{5,30})=12.68$, $p<0.0001$ *a*,*b*,*c* differ from the value of May for different indices at $p<0.05$, $p<0.01$ and $p<0.001$ respectively.*c* differs from the value of PF Score in September at $p<0.0001$ respectively .*e*,*f* differ from the value of BF Score in August at $p<0.05$, $p<0.01$and <0.001 respectively (Student's *'t'* test).

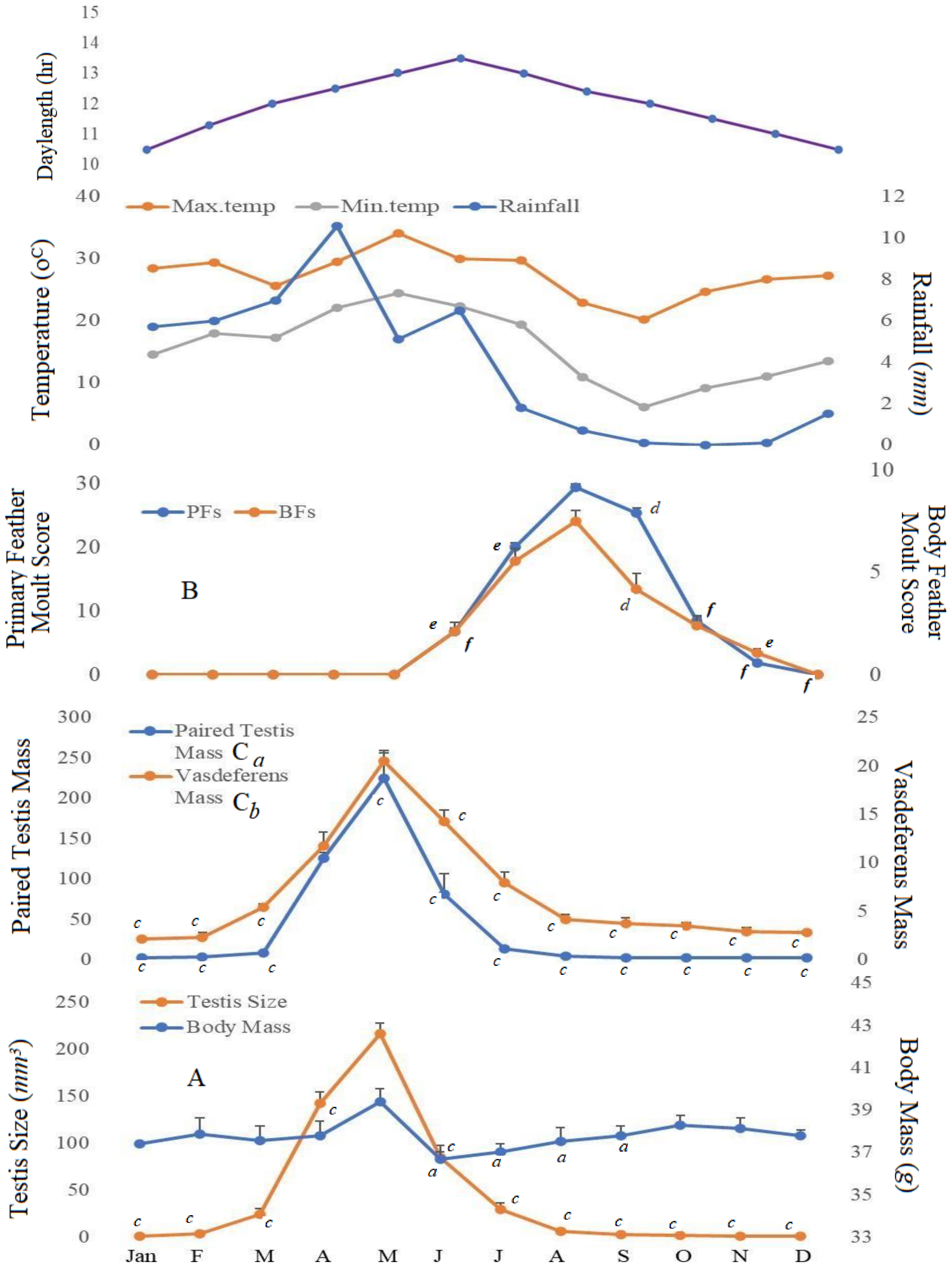

Fig.4: Seasonal changes in Body Mass, Testicular Size, Weight, Vasdeferens Weight and Moult Score of wild Red-vented bulbul, *Pycnonotus cafer stanfordi* at Lumami (26ºN). Values are Mean±*SE*, n=6, Body Mass: F($_{11,60}$)=2.22, $p<0.005$; Testicular Volume: F($_{11,60}$)=116.98,$p<0.0001$; Paired Testis Mass: F($_{11,60}$) =71.56, $p<0.0001$: Vasdeferens Mass: F($_{11,60}$)= 90.55 (ANOVA). Primary Feather Moult Score: F($_{5,30}$)=192.35, $p<0.0001$; Body Feather Moult Score: F($_{5,30}$)=19.86, $p<0.0001$ (ANOVA). *a,b,c* differ from value of May for indices within parameters at $p<0.05$, <0.01and <0.001 respectively. *d,e,f* differ from PF and BF Scores compared with August value at $p<0.05$, <0.01 and <0.001 respectively (Student's *'t'* test).

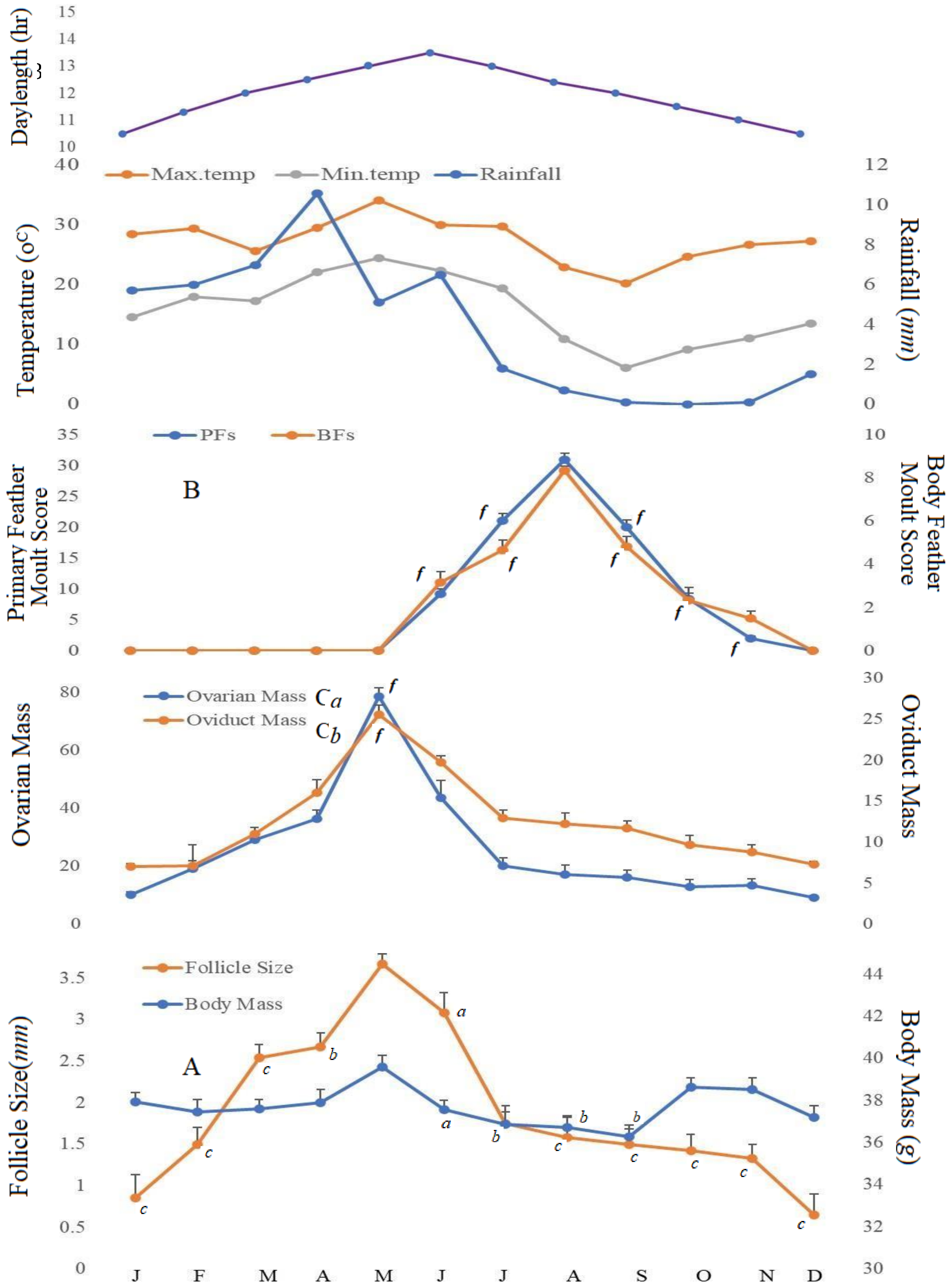

Fig.5: Seasonal Changes in Body Mass, Follicular Size, Ovarian Mass, Oviduct Mass and Moult Score of wild Red-vented bulbul, *Pycnonotus cafer stanfordi* at Lumami (26°N).Values are Mean±*SE*,n=6. Body Mass: $F_{(11,60)}=3.08$, $p<0.001$; Follicular Size: $F_{(11,60)}=33.74$, $p<0.0001$; Ovarian Mass: $F_{(11,60)}=50.11$, $p<0.0001$;Oviduct Mass: $F_{(11,60)}=56.32$, $p<0.0001$(ANOVA). Primary feather Moult Score: $F_{(5,30)}=69.71$, $p<0.0001$; Body Feather Moult Score: $F_{(5,30)}=26.17$, $p<0.0001$ (ANOVA). *a,b,c* differ from value of May within parameters at $p<0.05$, <0.01 and <0.001 respectively .*f* differs from the values of PF and BF Scores in August at $p<0.001$ (Student's *'t'* test)

in May and a minimum of 2.19±0.22 mg during November (Table 4, Fig.4Ca). Mean paired testis mass during the month of May was significantly more compared to values obtained in other months (Table 4, Fig.4 C*a*). Mean vasdeferens mass varied significantly ($F_{(11,60)}$= 90.55, p<0.0001) between a maximum of 20.50±1.07mg in May and a minimum of 2.80±0.15mg during December. Mean mass of vasdeferens during May measured significantly more as compared to values obtained in other months (Table 4, Fig 4C*b*).

Ovarian mass closely followed follicular cycle and varied significantly ($F_{(11,60)}$= 50.11, p<0.0001) between a maximum of 78.68 ± 3.02mg in May and a minimum of 9.18 ± 0.67mg in December. Mean ovarian mass in May was significantly more as compared to values obtained in other months (Table 5, Fig. 5C*a*). Mean oviducal mass differed significantly ($F_{(11,60)}$= 56.32, p<0.0001) across the season and measured a maximum (25.58±1.13mg) in May and a minimum (6.98±0.42mg) in January. Oviducal mass in May significantly differed as compared to values recorded for other months (Table 5, Fig. 5C*b*).

Histological Changes in Gonads: Seminiferous tubules in the testis were lined with single or double layers of spermatogonial cells during December/January (Plate Ia). During January-February seminiferous tubules increased in diameter and germ cell lines had advanced up to secondary spermatocytes as the most developed cell types (Plate Ib). In February-March there was sudden increase in the diameter of testis tubules and all the stages of the spermatogenetic cells including spermatids could be observed (Plate Ic,d). Seminiferous tubules increased further during April and May and contained bunched spermatozoa radiating centrifugally from the central lumen (Plate Ie,f) In June, diameter of seminiferous tubules started decreasing and degenerating spermatozoa along with desquamated cell debris were marked to advance towards central lumen of the tubule (Plate IIa). During August/September, seminiferous tubules decreased further and central lumens became filled with degenerating and desquamated germ cells (Plate IIb). Testis tubules became reorganised during the months of December with single or double layers of spermatogonial cells (Plate II e,f).

Ovary was dominated by the nesting oogonia and differentiating oocytes during December (Plate IIIa). Follicles began developing during December-January but had no clear distinction between thecal and granulosa cell layers (Plate III b,c). Thecal and granulosa cell layers became distinct as the follicles increased during March-May period (Plate III d-f). Follicular regression

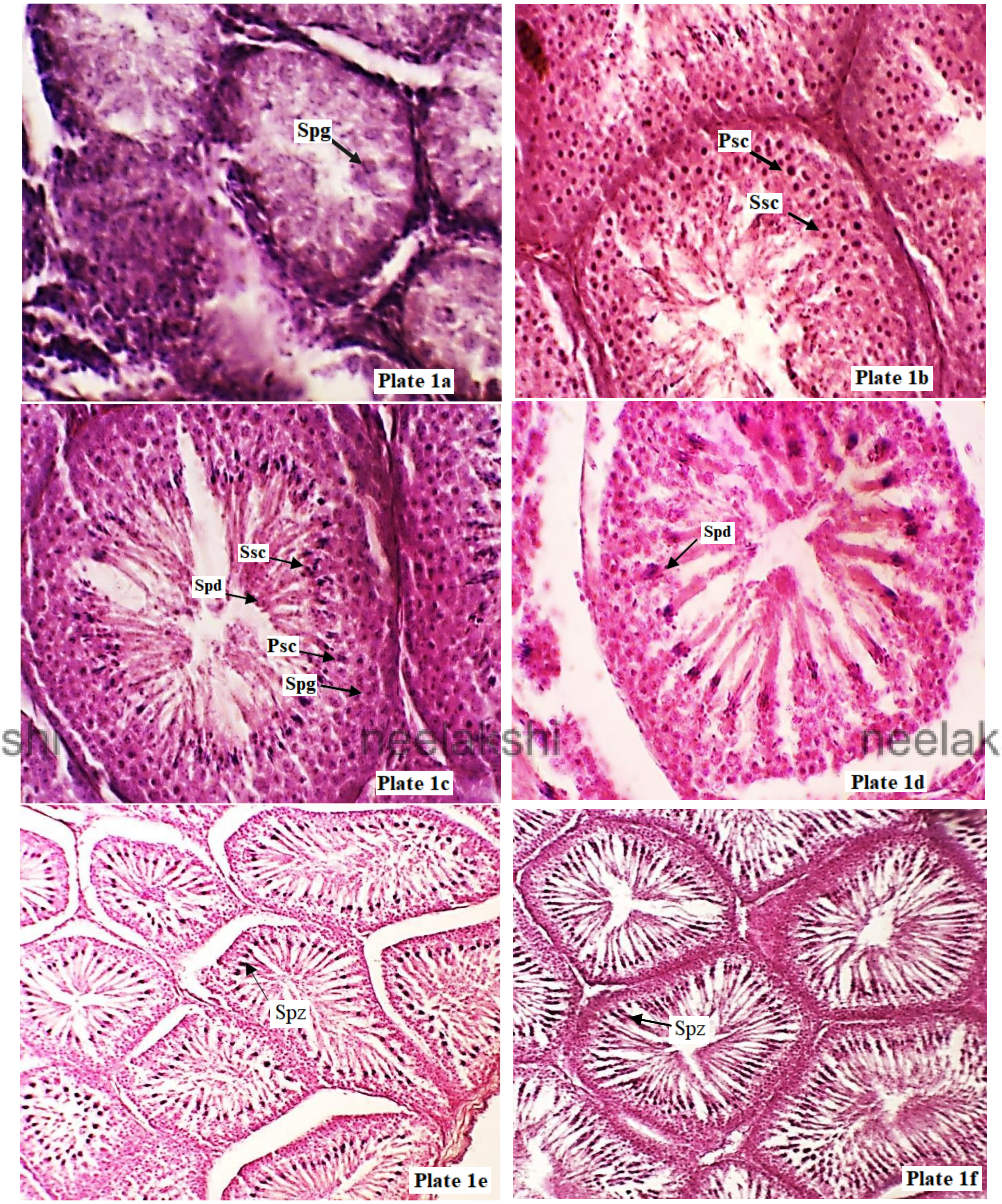

Plate-I a T.S. of quiescent phase testis showing seminiferous tubule containing single layer of spermatogonial cells (Spg) (Dec/Jan, x100); 1b seminiferous tubules showing presence of primary(Psc) and secondary spermatocytes (Ssc)(Feb/Mar, x100); 1c-d, T.S. showing germ cell lines advancing upto spermatid stage (Spd)(Mar, x40); 1e-f T.S. showing bunched spermatozoa(Spz)(May,x10).

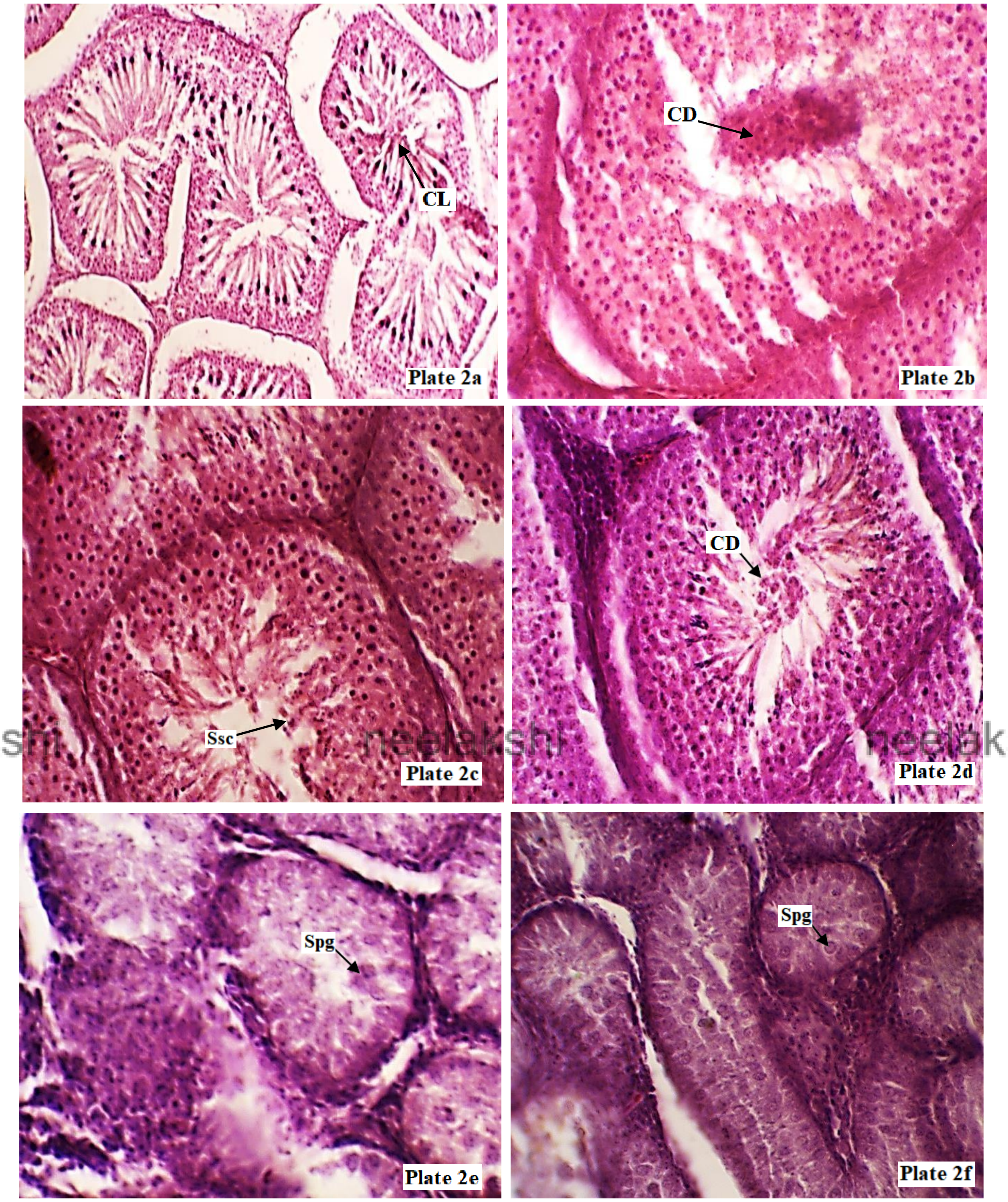

Plate-II a T.S. of testis in June showing shrinkage of seminiferous tubules and the start of degenerative changes.Germ cells heading towards central lumen (CL) x10; b Central lumen filled with degenerating tissue debris (CD) x40; c T.S. showing secondary spermatocytes (Ssc) as the most advanced cell types in thyroidectomized photostimulated birds x40; d degenerating germ cells in seminiferous tubules of thyroidectomized birds during April x40; e T.S showing reorganising germ cells lines during Dec x40; f T.S. showing reorganized tubules with spermatogonial cells during Dec x40.

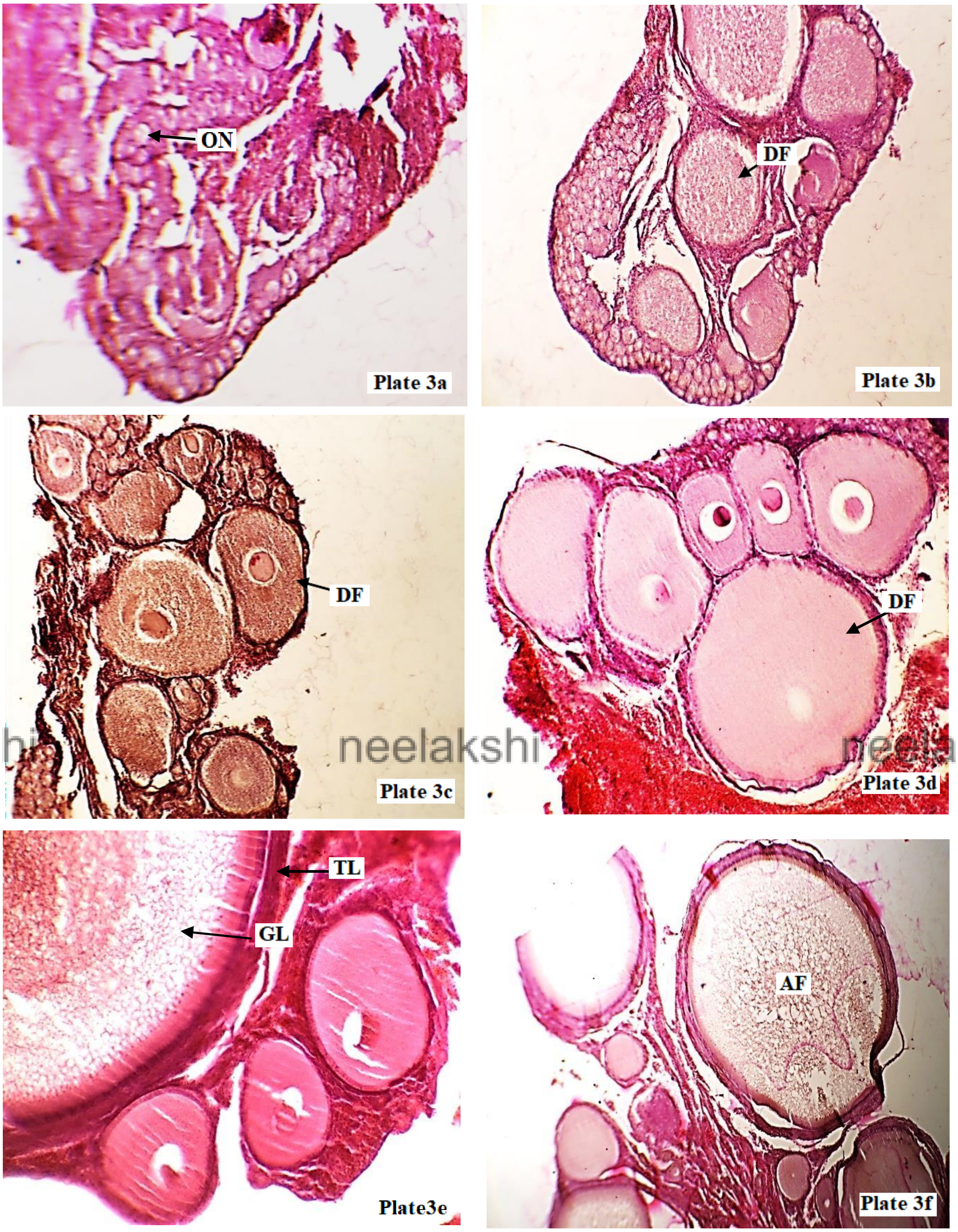

Plate III. Photomicrograph showing different developmental stages of ovarian follicles in Red-vented bulbul. a . T.S. of ovary showing oogonial nest (ON) in Dec x100; b-c. T.S of ovary with developing follicle (DF) in Jan-Feb x100; d. T.S. of ovary during Mar with developing follicle x40; e. T.S. of ovary showing developed follicle with distinct functional thecal (TL) and granulosa cell layers (GL) x40; f. T.S. of ovary showing atretic follicles during July x40.

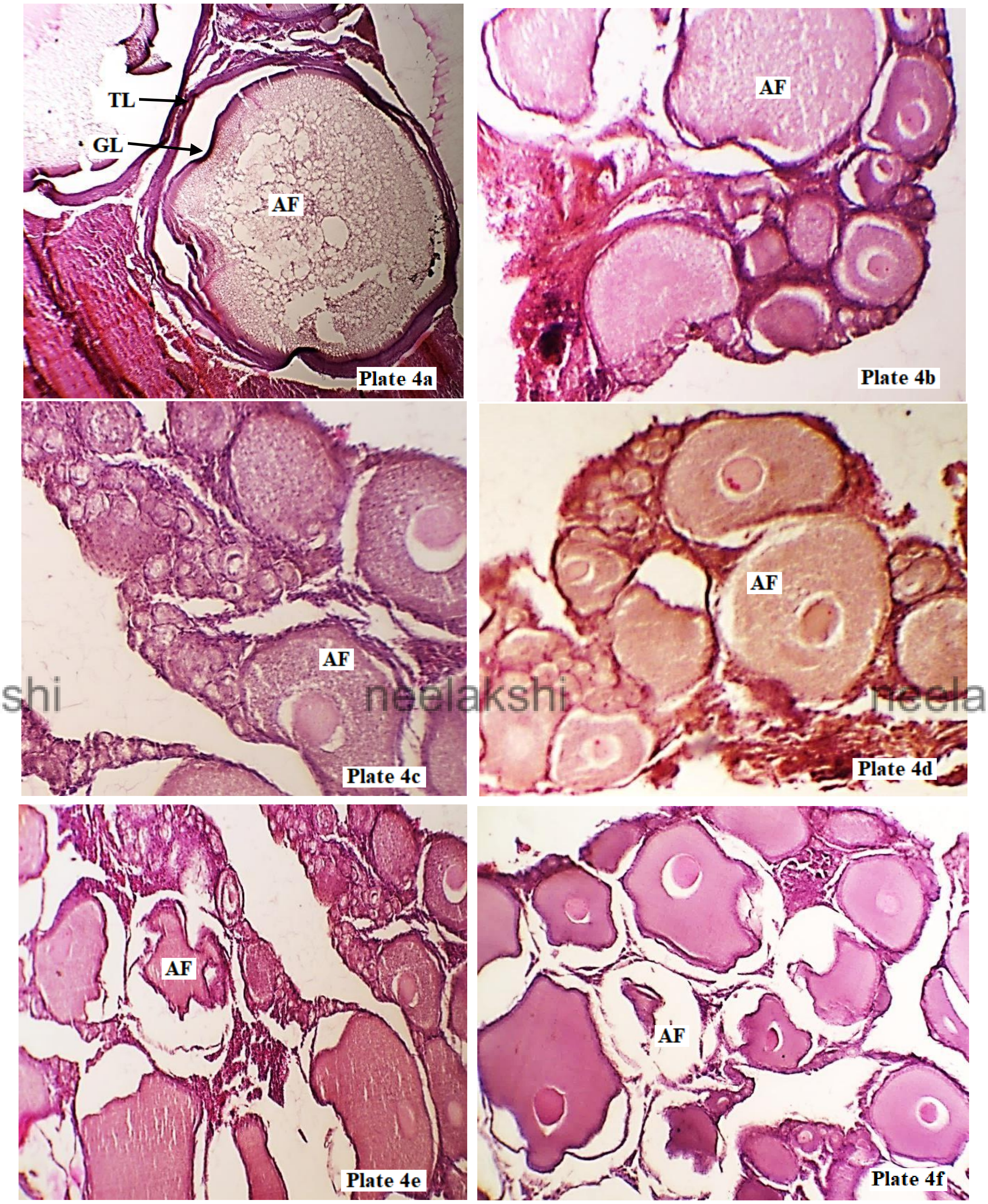

Plate IV. Photomicrograph showing different developmental stages of ovarian follicles in Red-vented bulbul. a .T.S. of ovary showing atretic follicle (AF) with degenerating TL and GL x40; b . ovary with atretic follicle in Aug x40; c-d Ovary containing atretic follicle and oogonial nest during Sept x40; e-f T.S. ovary sowing advanced stages of atretic follicle during Oct x40.

began in June with atretic follicles dominating the scenario (Plate III f). Follicles continued regressing through July/August and in September small atretic follicles were seen dominating the ovarian scenario (Plate IV a-f).

Seasonal changes in Body Mass: Body mass of both the sexes of red-vented bulbuls exhibited significant variations across the season (male: $F_{(11,60)}$= 5.44, $p<0.0001$; female: $F_{(11,60)}$= 3.04, $p<0.001$; One way repeated measures ANOVA). Body mass of captive males did not change appreciably from January-April period, reached at peak in May (37.75±0.47g) which was followed by a steady decline to reach seasonal low in July-August (35.38±0.34g). Body mass of birds in May measured significantly more compared with values from July-September months (Table 2, Fig. 2A). Significant increase in body mass of birds was registered during September-October which remained maintained until December (Table 2, Fig. 2A). Body mass of free living birds paralleled changes in body mass of captive males with maximum at 39.38±0.36g in May and minimum at 37.02±0.64g in July (Tables, 2, 4, Figs. 2A, 4A).

In captive females, body mass increased insignificantly from January-May and decreased during May - June period to reach annual minimum (36.07±0.47g) in July. From August, body mass began increasing and by November, mean body mass had significantly ($p<0.05$) exceeded the value observed in May (Table 3, Fig. 3A). Mean body mass of females in May and November were significantly more as compared to their values from June-August (Table 3, Fig. 3A). Seasonal changes in body mass of captive and free living birds were temporally similar (Table 5, Fig. 5A).

Seasonal Moulting Patterns: Feather moult was absent from January to May in both the sexes of birds maintained in the captivity and/or their counterparts in the wild. In captive male and female birds primary feather moult started in the month of June, increased through July-August, peaked in September and thereafter declined to end in the Month of November. In both the sexes, PFs moult scores varied significantly (males: $F_{(5,30)}$=36,26, $p<0.0001$; Females: $F_{(5,30)}$=87.77, $p<0.0001$) with their peak values at 30.00±1.27 in males and 31.72±0.89 in females in the month of September. In both the sexes of free living birds as well, PFs moult scores varied significantly in males ($F_{(5,30)}$=192.35, $p<0.0001$) and in females ($F_{(5,30)}$=69.71 $p<0.0001$) with peak values at 29.33±1.22 in males and 31.00±1.00 in females in the month of August. Body feather moult scores varied significantly in male ($F_{(5,30)}$=12.07, $p<0.001$) and

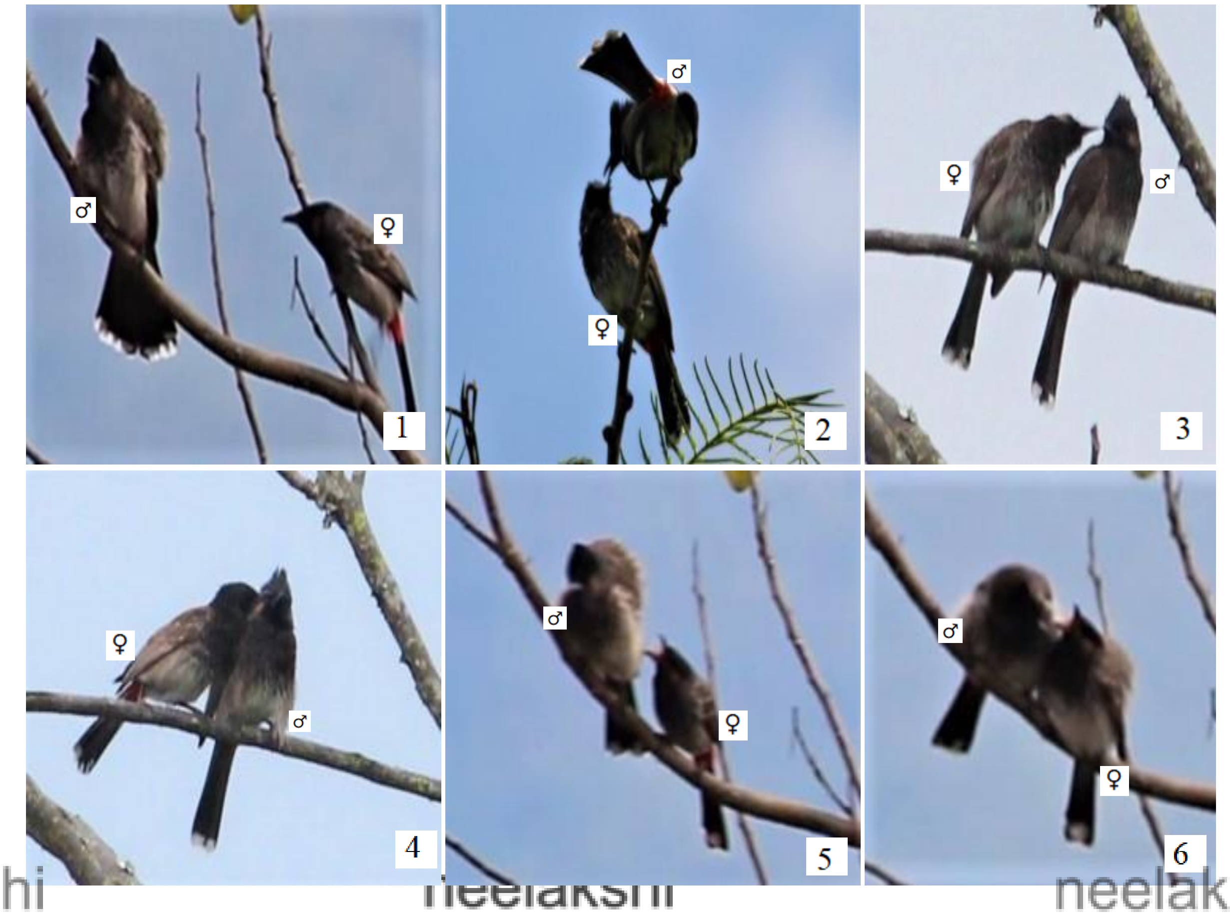

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Plate VI. Photograph showing courtship display and pair formation leading towards mating.

1.Female bird approaching male responding to his cooing.
2. Bill contact in male and female birds.
3. Pair of bird perching together and interacting with each other.
4. Female grooming male bird.
5-7. Female responses to male as a mate.
8.Female bird puffs her feather begging for mating.
9.Male attempts mounting the female bird.

female ($F_{(5,30)}=12.68$, $p<0.001$) captive birds with peak values at 6.27±0.61 in males and 7.259+±0.44 in females in the month of August. Similarly, significant changes in BFs moult scores of wild birds (males: $F_{(5,30)}=19.86$, $p<0.0001$ and females: $F_{(5,30)}=26.17$, $p<0.0001$) were registered with peak moult scores at 7.50±0.56 in males and 8.33±0.21 in females during August. Timings and trends for the onset and the completion of moult of PFs in both the sexes of free living and captive bulbuls were essentially similar (Tables, 2 – 5, Figs. 2B- 5B).

Expression of Reproductive Behaviour and Associated Physiological Attributes:

Courtship display, Pair-bonding and Nest-site selection: From November to February all the four (A-D) study segments were thinly populated by red-vented bulbuls. During March/April, population dynamics had suddenly changed owing to increase in the number of birds. With the influx of new populations in the study area, melodious chirping sounds of bulbuls from dawn to dusk became a pleasant attraction. In some resident bulbuls, courtship displays and pair-bonding were seen to commence at the earliest by the middle of February which intensified gradually keeping pace with changes in weather conditions, peaked by the middle of March to induce nesting during March/April. Around this timing, nomadic populations of red-vented bulbuls appeared in the study area and in them courtship display and pair-bonding occurred in quick succession followed by birds moving to discrete pockets of the habitat. This feature led to the culmination of second wave of nesting during May-June which involved larger chunk of bulbul populations in the study area. Further, during August-September period, fresh courtship displays were envisaged in some birds which entered into active nesting by the middle of September.

Courtship display and pair-bonding attributes were characterized by a minimum of 2-3 males competing to attract a female by perching closer and uttering a sequel of long, soft chirping calls of varying magnitudes. The cooing in male was followed by desperate wooing of the female to win her favour as a mate. The female succumbed to the preferred mate by gentle flapping of her wings (Plate-VI, 8-9). Pair-bonding was strengthened further by grooming the feathers of each other (Plate-VI, 3). During the course of mutual interaction, sexes were seen bringing their bills and feathers in contact (Plate-VI, 2 &4) and in the process, occasionally, their wings got interlocked making them rolling down the tree reaching up to the ground for few seconds before their wings became unlocked. This tendency could be seen happening occasionally for about a week during which pair-bonding was strengthened and birds left the

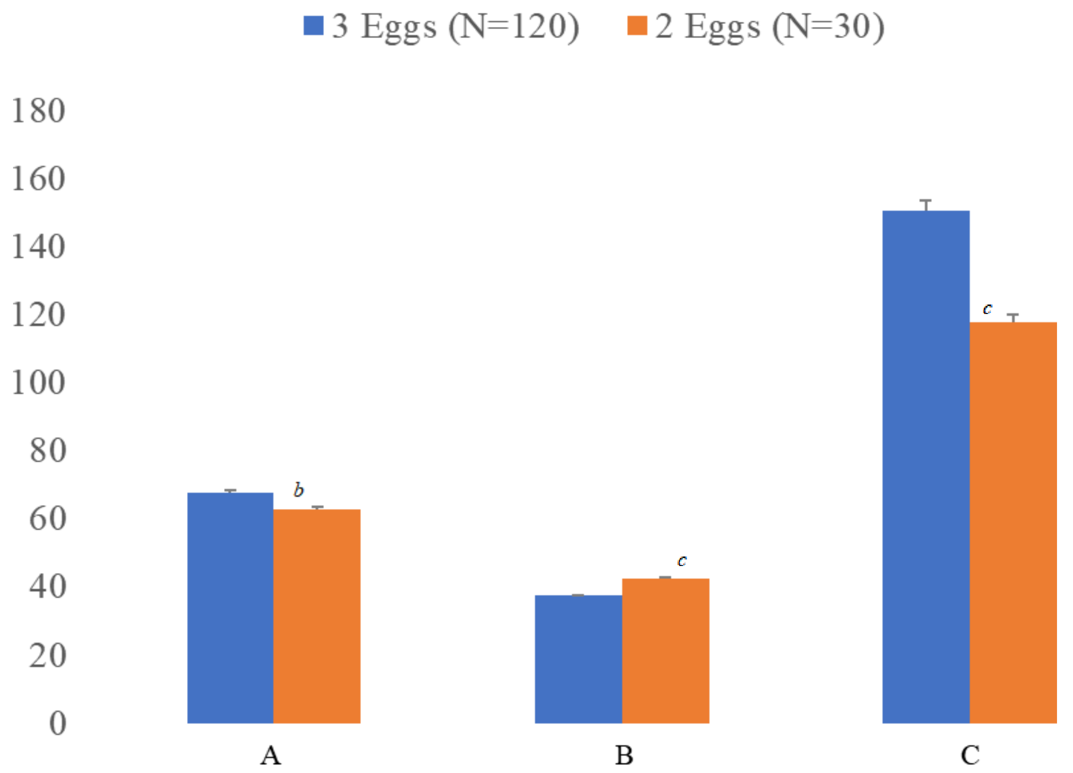

Fig.6: Diameter, Depth and Basement Area of the Nest of Red-vented Bulbul, *Pycnonotus cafer stanfordi*. A-Nest diameter at rim level; B- Total depth of nest; C- Basement area of the nest; Values are Mean±SE; *b,c* differ from the corresponding values of parameters as compared to nest having 3 eggs as the clutch size at p<0.05and p<0.001 levels respectively (Student's '*t*' test).

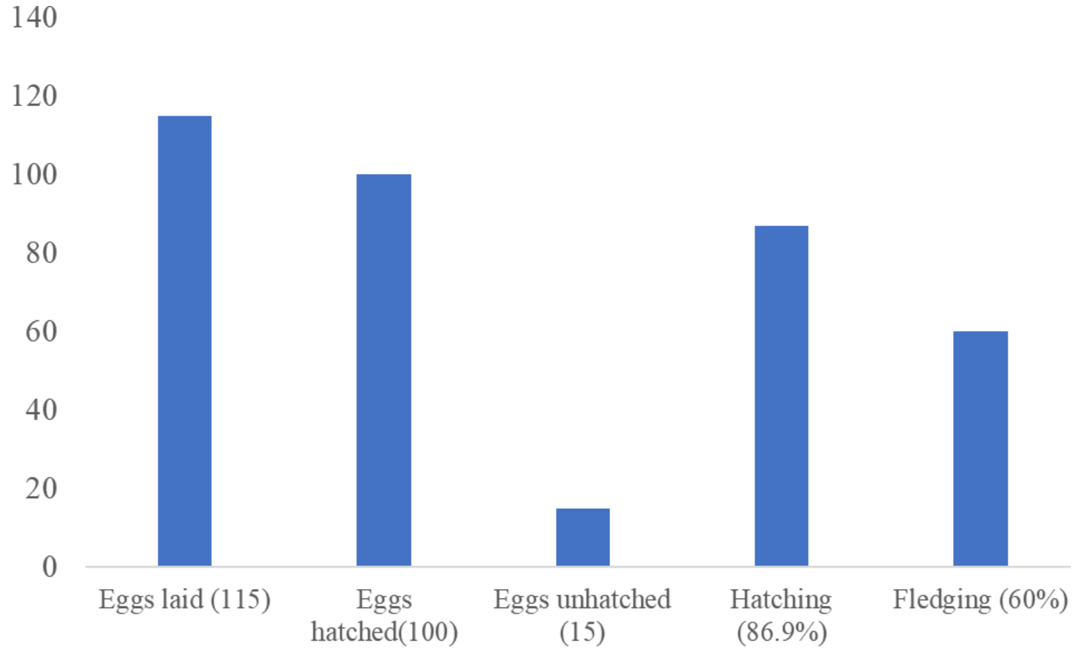

Fig.7: Breeding Success in Red-vented bulbul, *Pycnonotus cafer stanfordi*.

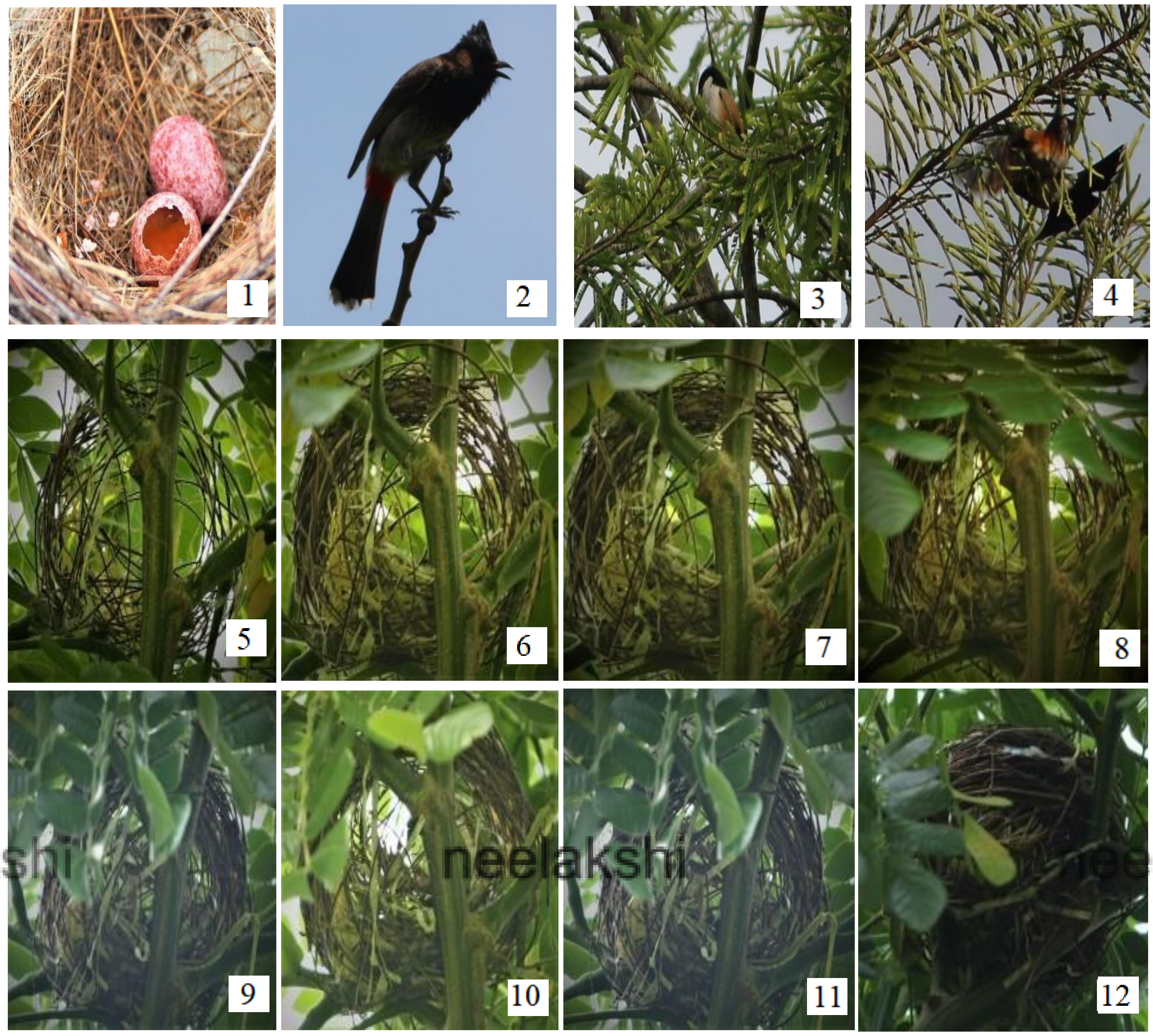

Plate VII. Photograph showing Territory protection and Nest construction process.

1.Eggs of bulbul sucked by long tail shrike during incubation
2. An alert male guarding territory against conspecifics and hetero-specifics.
3. A shrike (*Lanius schach*)encroaching bulbul's territory
4. A dreaded conflict between a shrike and red-vented bulbul. Shrike has over powered bulbul
5-6. Framing of nest-rim and subsequent adherence to host tree bough on day1
7-8. Enmeshing nest with thatch grass materials in the nest on day 2.
9-10. Further enmeshing of nest cage on day 3.
11. Enmeshed materials compacting from top towards bottom on day 4.
12. Nest bedded with soft leaf and flowers to complete nest on day 5.

group in search of suitable nesting site. The cascade of events involved birds visiting trees one after the other and exhibit intent hopping from one branch to the other in search of a suitable site for placing nest. Trees holding the remains of nests fixed in the preceding season were found to be preferred for fixing a fresh nest. Further, before the site for placing a nest was mutually determined, birds were seen to continue hopping around a forked-branch tilting and turning their body towards each other and exhibit motions characterizing building of a nest without any nesting material. This feature was found to be consistent amongst nesters. Small trees or bushes growing on the sides of open areas were found to be preferred for fixing nest.

2. Establishment of Territory: Once the site for fixing a nest was determined, birds became desperate to setup a territory by earmarking an area approximating 30 square meters surrounding the nesting spot. Pairs were observed vigorously defending their nesting-territory against con-specific and/or hetero-specific intruders. This tendency continued growing stronger amidst egg-deposition and incubation. Once hatchlings appeared in the nest, birds became extra cautious and profoundly aggressive in defending their territory against con-specific intruders and were often seen uttering continuous alarms (*phonetically sounding as twee twee twee*) with tough gesture to combat predators tiptoeing their territory. A dry vertical branch on the tree-top was usually engaged as the view point by the male bird to guard surroundings (Plate-VII, 2-4).

3. The Nest*:* Observations made on nesting behaviour of red-vented bulbuls from 2017-2021 in a stretch exceeding 5km^2 area in and around the headquarters of Nagaland University at Lumami, showed nesting season protracting from March/April to August/September with seasonal peak appearing during May/June. Of 300 active nests examined during the study period, 150 (50%) were found fixed in mango tree (*Mangifera indica,* from March-September), 90 (30%) in wild cherry (*Prunus avium,* during May - June), 25 (8.33%) in gulmohar (*Delonix regia,* from June-August), 20 (6.67%) in pride of India plant species, (*Lagerstroemia speciosa*, in May-June), 12 (4%) in bitter bean, stink bean or twisted cluster bean (*Parkia speciosa*, in June-July), and 3 (1%) in marigold or Mexican sunflower plants (*Tithonia diversifolia,* in June-July). Further, majority of nests were seen built lurking amongst deep foliage at the centre of the tree. No nest was found built at any human raised structure. Reminiscent expression of nest building behaviour could be envisaged manifesting in captive birds when nesting materials were available in the cage/aviary.

Cup-shaped nests were architectured generally at the centre of a multi-forked branch of the host-plant/tree at an approximate height of 2-6 metres from the ground. Nest construction began with the preparation of a 'rim' at the apex bordering the opening of the nest. The rim was carefully adhered to the fork using compact masses of spider webs (Plate VII, 1). Occasionally, birds were found injuring soft surfaces of the fork by their bills before sealing them with the nest-rim. Thin plastic or jute ropes were often used by the mates for tying nest with the host- branch. Leaf-rachis of gulmohar (*Delonix regia*) and morning glory (*Ipomoea hederifolia lutea*, *Ipomoea alba* and *Ipomoea indica*) vines were frequently used in making nest-rim. Dry pliable leaf-rachis of gulmohar and culms of thatch grass were curved in inverted dome-shape to connect opposite sides of the rim and then seal them using spider webs to create a lattice which was later filled with loose interlacing of dry leaf-blades and thin stems of thatch grass growing in abundance at the study sites (Plate VII, 5). In the following steps, enmeshed stuffs were slowly compacted starting from the top towards the bottom. The bottom of the nest was generally parked at the base of the fork and knitted with long leaf-blades, flower rachis and thin stems of thatch grass (Plate VII, 1). Upper side walls of the nest were knitted with small holes here and there.

Both the sexes of bulbuls were seen collecting nesting materials and arrange them to construct a cup-shaped structure. The female was found arranging nesting materials to shape the nest and periodically quivering to create a smooth cavity sufficient to accommodate brood and the mother. On clear sunny days, a nest could be made within 4.5±0.25 days, but when intercepted by incessant rain, the finish was delayed owing to the availability of dry nesting materials in the habitat. Recurrent insecurity posed by predators and/or hetero-specifics amidst nest construction prompted mates to abandon the nest unfinished. At no point of time, two active nests could be seen parked in the same tree. Measurements made at the rim-level from a pool of 150 nests showed an average thickness at 5.68±1.4 *mm* which decreased as the nest tapered downwards reaching 3.48±1.12 *mm* at the bottom. Average inside nest-diameter at the rim level was at 67.62±0.83 *mm* (nest with 3 eggs, n=120), 62.83±0.47 *mm* (nest with 2 eggs, n=30) and depth at 37.39±0.22 *mm* (nest with 3 eggs, n=120), 42.40±0.46 *mm* (nest with 2 eggs, n=30) respectively. The basement area of the nest measured at 117.80±2.10 cm^2 (nest with 2 eggs) and 150.34±3.15 cm^2 respectively (Table 6, Fig.6). Mean basement area of the nest with a clutch size of 3 eggs was significantly more ($p<0.001$) and depth significantly less as compared to the nest with 2 eggs only (Plate-VI, 5-12). Entry and exit routes to the nest appeared to be determined prior to their positioning at the host-branch.

4. Mating: Although mating pairs of red-vented bulbuls could be spotted from late March to mid-September, peak mating season clustered around May/June. The process began with male approaching female and attempt seducing her. Occasionally, female was also seen moving forward to reciprocate. Mutual consent culminating into mating was preceded by enticement of male by the female as a pre-requisite (Plate-VI, 9). During mating, female was found perching on a thin branch and by periodically slanting her body forward, she gave access to the male to mount her. At each mount, tilting his tail the male was seen transferring sperms into her genital tract. The mating was seen instantly repeating for at least 2-3 times.

5. Egg Laying, Clutch Size and Incubation: In red-vented bulbul, first egg was deposited within 24-36 hours following completion of the nest. During egg deposition, male bird was seen perching on a neighbouring branch keeping eyes on the female and the surroundings. Soon after depositing an egg, female came off the nest, stretched her wings and then together with male left for foraging. Eggs were deposited singly on consecutive days usually in the forenoon. The clutch size consisted of 2-3 eggs, oval in shape with ground pinkish colour and uneven dark red-spots all over the body (Plate-VIII, 2). Removing an egg from the nest soon after the second or third egg was laid or adding a conspecific egg to the nest immediately after the first or second egg was deposited, did not influence the final clutch size (Table 7). The average size and mass of an egg (N=300) was at 3772.56±54.65 mm^3 and 3.82±0.15g respectively (range: length, 21-24mm and width 15-18mm; mass, 3.25-4.12g, Table 8). Incubation commenced following the deposition of penultimate or ultimate egg of the clutch. Only single parent was found taking part in the incubation process and the other perching nearby was seen alert to chase away conspecific intruders in their territory. Incubating birds (n=15) caught during night on different occasions were subjected to laparotomy to ascertain their sex and all were invariably found to be female. Before start of incubation, birds were seen foraging for longer periods particularly at dawn and dusk. But, foraging timings had reduced slowly owing to incubation and very often, male was seen fetching a morsel of food for the incubating female, particularly on days when incessant rain had led to a sudden drop in the ambient temperature necessitating prolongation of incubation timings.

Plate VIII. Photograph showing chick-development stages of red-vented bulbul
1.A cup shaped nest; 2.Nest with a clutch size of 3 eggs; 3.Nest with incubating female.; 4. Hatchling appearing on 14th day of incubation. Chick starts begging for food on day 1;
5. Nest on day 2 holding 2 hatchlings with an unhatched egg.;6-10.Development of chick on days 3-11. Eyes remained closed with sprouting of feather papillae;11. Development showing opening of eyes on day 8 after hatching;12-14. Developmental stages showing rapid feather development on days 9-11.


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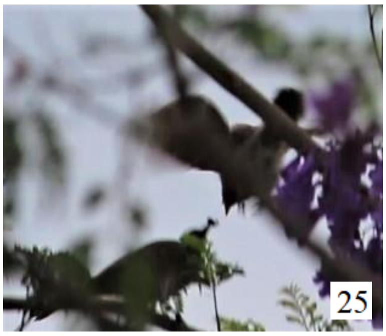
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Plate IX. Photograph showing chick development and parental care of fledglings in red vented bulbul

16-18. Developmental stages during days 12-14 of incubation. on day 14 young ones leave the nest.

19-23. Stages showing begging of nestlings and feeding with wild cherry fruit by parent bird.

24. Parent bird carrying preying mantis to feed nestlings.

25.Feeding of young one by parent bird with winged termite.

26. Showing skilling of young ones for catching food.

27. Young birds respond to parents’ call and position to evade predators. Two fledglings sitting in bush with heads buried in feathers.

Incubation was continuous during night (~18.30 hrs - 4.30 h, n=10). However, during day time parents were observed leaving nest intermittently for foraging with hourly frequency at 2.65±0.90. On the average, female spent 63±13% of natural lighting hours on incubating. An average on-bout (n=10) and off-bout (n=10) timings were at 15.54±8.50 min and 12.51±5.10 min respectively. Hatching asynchrony was observed in 69.56% cases, synchronized hatching occurred in 17.39% eggs and the remainder 13.05% eggs failed to hatch (Table 9, Fig.7). On clear sunny days, hatching was complete within 14±0.11 days (n=40). But, when intercepted by inclement weather, incubation period was prolonged by a day or two.

6. Hatching and Parental Care: Eggs were found to hatch one by one at a gap of 24-30 hours. Nestlings emerged bare bodied with their eyes closed but with functional acoustic perceptions. Female continued sitting in the nest to deliver hatchlings due warmth and both sexes were found caring for nestlings against con-specific and hetero-specific intruders and/or predators. Parental care began concurrent to the deposition of first egg in the nest. The stage was characterized by birds sounding alarms at the entry of an intruder and/or predator within their territory. This tendency continued growing stronger with more eggs added to the nest. Towards completion of the clutch size, birds became extra vigilant and acquired inflexible tendency to resist unpleasant distress posed by conspecific or heterospecific encroachers of their territory. Parental care heightened with first hatchling appearing in the nest to pose obligatory feeding requirements within an hour after hatching and to continue till chicks had grown to fend for themselves. Both the sexes of bulbuls were seen fetching soft morsel of food comprising chiefly of larvae of moths and butterflies, insects (grass hoppers, aphids, thrips, ants and winged-termites *etc*), spiders, nectar, small flower buds and fruits (banana, papaya, guava, cherry, mulberry, black plums, black nightshade and velvetleaf fruits *etc*) to nourish young ones. Parents were observed nourishing chicks by turns. Soon after feeding chicks, incumbent bird was seen leaving nest signalling his partner to take her turn for feeding. For about a week eyes remained closed, hatchlings could smartly guess at parent's visit to the nest as they were found instantly opening their mouth for a gape and continued begging till rewarded with a morsel of food (Plate-IX, 15-23). Gaping behaviour could be simulated in nestlings by gentle patting at the nest (Plate-VIII, 4, Plate-IX, 5, 6 & 21). Recurrent perturbations in the habitat, owing to anthropogenic activities and/or enhanced predation pressure amidst hatching, heuristically occasioned mates to heighten

Plate X Photograph showing murmuration in combined flock of red-vented and black-headed bulbuls

1.Flying squad starts forming at the behest of call from roost leader (shown by red arrow).

2. A flying squad with several hundred birds photographed on 12th of October 2019 during morning hours at university campus, Lumami (26° N).

security concerns and a consequent drop in the feeding timings resulted rejection of chicks born late.

Development of Chicks: Female continued spending night with nestlings. Feather papillae appeared on day 4-5 (Plate-VIII, 7) and plumages began sprouting on day 6-7 (Plate-VIII, 11). Eyes opened on day 7-8 of incubation (Plate-VIII, 11). As the eyes opened, chicks were noticed exhibiting fear complex. The stage was characterized by nestlings start moving their wings and making noises louder than before to attract attention of their parents. Between days 10-13, nestlings became fully covered with feathers (Plate-IX, 16-18), started fluttering their wings and were seen struggling to hop out of the nest. Parents kept removing excretal wastes of young ones from the nest. Birds were seen strategically adjusting feeding patterns to make nestlings grow synchronously and be able to leave the nest together by day 14. However, very often, the youngest fledgling was found leaving nest delayed by a day or two. Nest was abandoned with all the chicks fledged. Slash and burn cultivation practices, indiscriminate killing of birds and high incidence of lifting grown up nestlings by the natives pose serious threat to the decline in the population size of red-vented bulbuls.

Skilling the Young: Fledglings kept wandering aimlessly risking them to predation. Parent birds continued with nourishing fledglings and guide them to bushy sites having less frequent human interference. As the young ones grew to hopping stage, they were engaged with training schedules developed by parent birds. Parents generally guided them to nearby fields under moderate grass cover and sparsely growing bushes. Training was imparted daily for about an hour, preferably at dawn and dusk. The process sparked with parents catching insects for obligatory feeding of young (Plate IX, 25-27). This prompted young birds to instinctively hustle to grab insects for self nourishment. After about half an hour, parents guided young birds to the nearby bush and parking them there, they moved around in search of food to fend for themselves. Interestingly, during this period, parents and the young ones remained at acoustic linkages and very often one of the parents visited them with a morsel of food. Occasionally, sensing the presence of predators around, parents were seen alarming young ones to hide from the sight of predators. Youngs were noticed instantly pushing them to a corner and sit ideal with their heads hiding under wing-feathers until parents arrived to escort them (Plate IX, 27). Training schedule was noticed to continue for about two weeks to make young ones fully equipped with tricks for acquiring food and evade predation. During night, parents and young birds were seen roosting together in bushy trees until such times they became part of a larger foraging flock.

Murmuration Display: During the study period (2017-2021) at Lumami (Lat. 26^{o}13' N, Long. 94^{0}28' E), bulbul murmurations were noticed occurring frequently during autumn months (late August to October). During the last week of August, distinct groups of red-vented, *Pycnonotus cafer standfordi* and olive and greyish forms of black-headed bulbuls, *Pycnonotus atriceps* or *Brachypodius melanocephalos* amassed to forage together and shared a common roost. On 12th of October 2019, a massive murmuration event was recorded in the morning hour (~6.30 am, (Plate X, 1, 2) when several hundred bulbuls (>1000 birds) foraging in discrete pockets of the habitat huddled to congregate at the communal roosting site in the hills bordering university campus in the south-west. The incidence was sparked by a single bird which began hovering over the communal roost discharging uninterrupted acoustic signal arousing others to follow, and in no time, several smaller groups joined to form a massive flock flying in synchrony above the roost. Periodically, groups were found to scatter and quickly reunite. The ever changing patterns of swirling and swooping of groups passing over and closer to their roost provided fair opportunity to the participants for intermittent exit and/or entry to the aerial show. This exercise was witnessed to repeat in surges of 3-5 episodes for at least 3-5 consecutive days in the morning hours around 6.30 a.m. lasting for about half an hour before the flock finally funnelled down to the roosts.

DISCUSSION

Food and Feeding Ecology

Present observations show that red-vented bulbuls consume a large variety of plants and/or plant products and invertebrate food resources to match their daily and/or seasonal calorie requirements. Flower parts and their products, such as nectar, petals, buds, fruits and seeds constituted the larger part (>77%) of total food consumed across months except that during post-nuptial period invertebrate food resources had increased in the gut contents as compared to other months. This fact was evidenced in the observation that between June and October/November animal food resources made up to 35% of the total food consumed. Thus, present findings corroborate generally agreed view that red-vented bulbuls are omnivorous and consume both, the large variety of fruits and insects (Bhatt and Kumar, 2001; del Hoyo *et al.*, 2005; Thibault *et al.*, 2018a,b, 2019; Nowakowski and Dulisz, 2019; Chishty *et al*, 2021; Zohaib *et al.*, 2021). Findings suggest that from November-February, plant resources (guava, flower buds, nectar, Mexican sunflower seeds) and animal nourishments (ants, honey bees and flies) constituted the major part of stomach contents. It is worth mention that during this period, the study area had witnessed a bloom of wild cherry which made major source for nectar collection by birds and honey bees. Red-vented bulbuls were found to synchronize their morning visits (~ 6.30 -7 am) to the flowering cherry trees with honey bees and in the process of nectar collection they often consumed plenty of hymenopterans. This tendency, though with lesser intent was also visible in day time (~1.30-2pm) and during evening hours (4-5pm). Similar scenario had surfaced during spring and summer months in the process of nectar collection from flowering coral trees like *Erythrina variegata*, *Erythrina indica* and Dalchini, *Cinnamomum verum* (also see Raju *et al.,* 2004). During March-May period, major parts of gut contents consisted of plant materials and/or their products like nectar, fruits (banana, *Musa acuminata*, jamun, *Syzygium cumini*, guava, *Psidium quajava*, papaya, *Carica papaya*, lichi, *Lichi chinensis*, banyan, *Ficus benghalensis* figs, mulbery, *Morus nigra*, goose berry, *Phyllanthus emblica*, Cornelian cherry, *Cornus mas*, Australian cherry, *Syzygium paniculatum*, wild cherry, *Prunus aviam*, plums, *Prunus domestica*, blackshade berry, *Solanum nigrum*, ivy gourd, *Coccinia grandis*, velvetleaf fruits, *Abutilon theophrasti*, lantana, *Lantana camara* nectar, berries and seeds, bottlebrush, *Callistemon* species pollen and nectar, *Erythrina variegata* and *Erythrina indica* nectars) along with mosquitoes, ants, cicadas, grass hoppers, thrips, aphids and termites *etc*. However, from June to October/November, unlike other months, gut contents had significantly more quantities of invertebrate food resources which consisted of spiders, cicadas, gross hoppers, ants, winged termites, besides nectar, small flower buds and fruits (banana, papaya, guava, plums, black nightshade and velvetleaf fruits *etc*). During this period, red-vented bulbuls were often seen competing with swallows to grab insects making aerial dives shooting up to 30 feet off the ground. Increased intake of animal food resources during post-nuptial phase may thus be accounted to the rich availability of insect-fauna in the habitat and the augmented metabolic needs towards calorie rich food resources to support rearing of young ones, repletion of energy stocks depleted during intense breeding activities and feather renewal which occur concurrently. Present observations do not support findings of some workers that red-vented bulbuls feed on house lizards, *Hemidactylus flaviviridis* (Sharma, 2000; Nowakowski and Dulisz, 2019). The fact that house lizards or their body-part remains could not be recovered in the gut-contents supports this view. The probable disparity might result due either to non-preference for house lizards by *Pycnonotus cafer stanfordi* unlike their sister subspecies or that birds have no access to this reptile for not having approximation with the human habitations. Further, notwithstanding the types and/or quality contents of plant and/or animal food resources across habitats, present observations favour the general assumption that red-vented bulbuls are predominantly frugivorous birds but they do exhibit distinct seasonal diversity in their food and feeding ecology (Bhatt and Kumar, 2001; del Hoyo *et al*, 2005; Brooks, 2013; Thibault *et al*, 2018a,b, 2019; Nowakowski and Dulisz, 2019; Zohaib *et al*, 2021).

Food and foraging ecology of red-vented bulbul, *Pycnonotus cafer stanfordi* causes pollination of a large variety of plants during the course of nectar collection. Consumption of ripe fruits of jamun, *Syzygium cumini*, guava, *Psidium quajava,* banyan figs, *Ficus benghalensis,* mulbery, *Morus nigra*, goose berry, *Phyllanthus emblica*, Cornelian cherry, *Cornus mas*, Australian cherry, *Syzygium paniculatum*, wild cherry, *Prunus aviam*, blackshade berry, *Solanum nigrum*, ivy gourd paves way for seed-dispersal of these valuable plants/trees. Intake of insects and their larvae may be interpreted as natural biological control mechanisms towards regulation of insect pests in the crop fields as undeclared blessings from bulbul populations to their farmer friends. Further, the contention that red-vented bulbuls cause significant damage to the orchards (Bhatt and Kumar, 2001; Thibault *et al*, 2018a,b, 2019), could not be noticed as a worrisome concern as no appreciable damage to fruit and vegetable crops could be noticed in the present study. When viewed together with the presence of vast reservoir of plants and invertebrate food resources in the wild, it may not be speculative to assume that when alternate food resources are available in the habitat, bulbuls cause least damage to the orchards. Dispersal of noxious plants like lantana, *Lantana camara* and Mexican sunflower, *Tithonia diversifolia* add to the darker side of the story. By the same token, deliberate consumption of hymenopterans by red-vented bulbuls during the process of nectar collection from flowers forms the major setback to the apiculture industry. Slash and burn practice in the area, deliberate hunting and lifting the grown up nestlings by natives around present some of the most appalling feature shrinking population size of red-vented bulbuls.

Gonads

Results show that gonads of red-vented bulbuls remained small and inactive during November-December/January (preparatory phase), began developing in January/February (early progressive phase), grew rapidly through March-April (late-progressive phase), peaked in May/June and thereafter declined through July-August (regression phase) to reach a small size in October-November (quiescent phase). Owing to the fact that gonad development cycles are temporally positioned to the solar cycle, it may be suggested that seasonal fluctuations in daylengths might become the key regulator of breeding cycles in red-vented bulbul. Gonadal recrudescence commencing during January/February may denote that critical daylength necessary for the activation of neuroendocrine-gonadal axis in the red-vented bulbuls might exceed 11 hrs. When viewed together with published literature on breeding performance of red-vented bulbul, *Pycnonotus cafer* from current distribution (30^{o}N-36^{o}S) within and outside its native ranges (McCann, 1931; Dutt, 1932; Lamba, 1968; Vijayan, 1980; Bhatt and Kumar, 2001; Walker, 2008; Acharya and Vijayan, 2009; Balkrishnan, 2010; Prajapati *et al*, 2011; Rao *et al*, 2013; Awais *et al*, 2014; Radhakrishnan and Ashokan, 2014, 2015; Yadav *et al*, 2018; Thiabault *et al*, 2018a,b, 2019; Zohaib *et al*, 2021), it may be suggested that timing and duration of breeding season in this species may vary depending on the latitude or the location but common feature entails breeding activities to begin only when prevailing daylength exceeds 11 hrs/day. It is noteworthy that distribution of *Pycnonotus cafer stanfordi* is restricted to the geographical ranges lying between 23^{o}N-29^{o}N where longest daylengths vary from 13.5-14hr/day. The fact that testes of red-vented bulbuls developed under simulated long daylengths (15L/9D and 16L/8D) and that constant short days (8L/16D and 9L/15D) prevented testicular recrudescence supports this view (Lal and Thapliyal, 1982a, also see Chapter-III). Spring and/or summer daylengths have also been shown to trigger precocious gonadal maturation and development of accessory and secondary sexual characteristics in a vast majority of avian species nesting at mid and higher latitudes (Wingfield, 1983; Sharp *et al*, 1986; Nicholls *et al*, 1988; Thapliyal and Gupta, 1989; Wingfield and Farner, 1993; Dawson, 2015; Ubuka *et al*, 2013; Nishiwaki-Onkawa and Yoshimura, 2016; Dixit and Byrsat, 2018). Progressive increase in natural lighting hours and parallel development of gonads between February and May might denote direct stimulatory impact of increasing daylengths on neuroendocrine-gonadal axis. It is however, intriguing to observe that in both the sexes of wild birds, absolute enlargement of gonads had exceeded by approximately 40% as compared to their counterparts maintained in the captivity. When viewed together with no significant difference in absolute body mass of wild and captive bulbuls, it may be argued that factors other than food supply might account for significant reduction in absolute development of gonads under captive conditions. Captivity stress may be accounted as one of the probable factors since it is known to trigger augmented release of endogenous opioid peptides which in turn, increase body weight but suppress gonadotrophin releasing hormones and/or gonadotrophins in birds and mammals (Millan and Hertz, 1985; Sakurai *et al*, 1986; Ebling and Lincoln, 1987; Stansfield and Cunningham, 1987a,b; Lal *et al*, 1990). Suppressed growth of gonads under captive conditions has also been reported in spotted munia, *Lonchura punctulata* (Vidyarani and Lal, 2007; Lal *et al*, 2012), Tree sparrow, *Passer montanus* (Lal *et al*, 2007; Dixit and Singh, 2011) and spotted doves, *Streptopelia chinensis* (Malik and Lal, 1998); Lal *et al,* 2013).

Decline of gonads during May-June in the face of naturally increasing lighting hours, might denote the development of photorefractory states - gonadal and metabolic. Such a supposition stems from the fact that during May-June, body mass of birds had also decreased and degenerative changes in germ cell lines were accompanied with significant decline in the weight of gonads and gonoducts. Thus, it may not be speculative to deduce that stimulatory impact of long daylengths on the neuroendocrine-gonadal and neuroendocrine-metabolic axes begins to breach during May-June owing to the development of photorefractoriness. Further, sustained gonadal regression in birds transferred from natural lighting condition to constant 16L/8D or 20L/4D in the month of June onwards (see Chapter III), lends support to this suggestion. Development of Photorefractory states- gonadal and metabolic- in photoperiodic birds have been viewed as physiological adaptations to divert attention towards caring the youngs and replenish energy reserves depleted during intense breeding activities, moult and migration which occur concurrently in quick succession (Wingfield, 1983; Sharp *et al,* 1986; Nicholls *et al,* 1988; Thapliyal and Gupta, 1989; Wingfield and Farner, 1993; Dawson, 2008; Ubuka *et al*, 2013). Natural decrease in lighting hours during autumn and winter months might account for dissipation of photorefractoriness and resumption of response to stimulatory daylengths; a typical characteristic which majority of photoperiodic birds heuristically exhibit.

Gonoducts Mass

The observation that vasdeferens and oviduct weights during different months closely correlated with the weight and not with the size of gonads, suggests that gonoducts mass is under the direct influence of circulating levels of sex steroids. By the same token, it may be inferred that gonoducts mass might serve as more reliable indicator of gonadal function than the size of the gonads. Simultaneous decline in gonads and gonoducts mass associated with degenerative changes in histological view of the gonads during May-June without appreciable changes in their size, advocate in favour of such a supposition. The fact that in some Indian finches, castration leads to reduction and exogenously administered sex steroids result full recovery of gonoducts weight (Thapliyal, 1981), supports this view.

Body Mass

Present findings show that in both sexes of red-vented bulbul, significant alterations occurred during the course of annual breeding cycles and their maxima and minima temporally coincided with gonadal peaks and troughs. Identical trends in body mass changes of wild and captive bulbuls suggest that the species has evolved intrinsic mechanisms to economize its energy resources. Present results seek conformity with the report of Lal and Thapliyal (1982a) who opined the lack of a body weight cycle in red-vented bulbuls maintained under captive conditions at Varanasi (25^{o}N). However, a careful insight into results presented by these authors in the said study, shows birds having maximum body weight in the month of May/June which was significantly ($p<0.01$) higher as compared to the mean values of August and September in both the sexes and thus fall in line with the current assumption. Furthermore, in the present case, full recovery in body mass during the post-nuptial period is at variance with the observation of Lal and Thapliyal (1982a), wherein post-nuptial recoupment of body weight was not apparent in captive females. It may be mentioned that differences in the habitat quality, nourishment types and the sub-species status alone or in combination, might account for differences in response of metabolic mechanisms to varying environmental cues. Interestingly, distribution of *Pycnonotus cafer stanfordi* subspecies of red-vented bulbul is limited to north-eastern parts of Indian sub-continent (southern hilly part of Assam, Arunachal Pradesh, Nagaland, Manipur, Meghalaya, Tripura, Bangladesh, northern segment of Myanmar and south-western parts of China (ZSI, 2006). No such studies have ever been undertaken from the entire stretch of its current distribution.

In many passerine and non-passerine birds sufficient energy stocks are made prior to the breeding season to meet eventual calorie requirements during the course of maturation of the reproductive system and discharge of post-breeding parental responsibilities amidst plumage renewal. Birds like pheasant, *Phasianus colchicus* (Clarence *et al*, 1962), black-headed munia, *Munia malacca malacca* (Thapliyal and Pandha, 1967a), lal munia, *Estrilda amandava* (Thapliyal and Pandha 1967c; Thapliyal *et al*, 1991; Lal and Thapliyal, 1995), chest-nut bellied munia, *Munia atricapilla* (Thapliyal and Garg, 1967; Thapliyal and Bageshwar, 1970), spotted munia, rook, *Corvus frugilegus* (Lincoln *et al*, 1980)), *Uroloncha punctulata* (Vidyarani and Lal, 2007; Lal *et al*, 2012), white-winged black bird, *Agelains phoeniceus* (Robinson and Rogers, 1979), Indian spotted dove, *Streptopelia chinensis* (Malik and Lal, 1998) and tree sparrows, *Passer montanus* (Lal *et al*, 2007) significantly higher body mass precedes breeding season. Increase and decrease in body mass of red-vented bulbuls concomitant with gonadal growth and regression, suggests that neuroendocrine-gonadal and neuroendocrine-metabolic axes are engendered by some common physical and/or physiological factors. Further, parallel increase and decrease in body mass and gonad size of birds prompts speculations about direct involvement of sex steroids in influencing the body mass cycles of red-vented bulbuls. This, however, seems contentious owing to full body mass recovery during autumn months in birds with small and inactive gonads. Influence of castration on seasonal changes in body mass of red-vented bulbul remains to be investigated. Body weight of spotted munia was not influenced by excision of gonads or replacement therapy with exogenous treatments of sex steroids (Thapliyal, 1968). This alone may not however, be adequate to negate a role of sex steroids in the regulation of body mass cycles of red-vented bulbuls. In a detailed study made in spotted munia, Gupta and Thapliyal (1984) have shown that influence of gonadal steroids on body weight of birds depends upon the doses employed and the reproductive status of birds at treatment. Few reports have presented convincing results to acclaim involvement of sex steroids in inducing pre-migratory fattening of birds (Weise, 1967; Tewary and Kumar, 1981; Thapliyal, *et al*, 1983), and the effects appeared to be caused through increased secretion of hypophyseal Prolactin (Yokoyama, 1976, 1977). Exogenous treatments of Prolactin are reported to cast profound influences on gonads and body weight of both, resident and migratory avian species Chandola and Pavgi, 1979; Thapliyal and Lal, 1984a; Seiler *et al*, 1992; Thapliyal, 1992).

Significant decrease in body mass of bulbuls between May and August might result due to development of metabolic refractoriness and a consequent drop in voluntary food intake amidst intense parental duty and feather renewal. Obligatory calorie requirements towards rearing young ones and concurrent accomplishment of feather renewal might thus necessitate rapid depletion of body reserves and a consequent loss in body mass. In sharp contrast, body mass repletion between September and November might result due to dissipation of metabolic refractoriness and consequent rise in voluntary food intake. It is significant to emphasize that body mass recovery period in the present case coincided with the flourishing of insect fauna in the habitat and that during this phase birds feed voraciously on insects and their larvae which constitutes the favourite food of both, parents and the young ones of red-vented bulbuls (Neelakshi, unpublished data). Seasonal changes in feeding choices, availability of quality food resources, diurnal feeding trends and total daily food intake immensely influence the body weight of birds (Farner, 1975; Chandola *et al*, 1980; Thapliyal, 1992).

Moult

Absence of moult in the wing primaries (PFs) and/or body feathers (BFs) from January to May denotes lack of a pre-nuptial moult in the red-vented bulbul. In many avian species, feather renewal occurs twice annually, first with less intense pre-nuptial moult that establishes sexual dimorphism by distinguishing males to wear brilliantly coloured plumage to help attract the mates. In such species, second moult occurs during the post-nuptial period and is relatively much more intense and abolishes sexual dimorphism by inducing regeneration of henny feathers (Thapliyal, 1981). Maintenance of flight efficiency, in essence, demands replacement of old and/or damaged feathers by new ones and in small birds this occurs at least once every year (Ginn and Melville, 1983). In the present study, initiation of moult in wing and body feathers of both, wild and captive birds during June closely correlated with gonadal decline might denote a functional link between gonadal steroids and the feather renewal process. Progressive increase in shedding of wing and body feathers during June-July with peak expressing in August/September and completion of moult by October/November closely associated with declining gonads strengthens this view. Onset of moult concomitant with the termination of reproductive activities is also prevalent in many other avian species nesting at sub-tropical and temperate latitudes (Thapliyal, 1997; Svensson and Hedenstrom, 1999; Dixit and Singh, 2011). Further, decline in gonads in the face of increasing natural daylength during May-June, coinciding with initiation of feather loss prompts the assumption that moulting process might result due to insensitivity of the neuroendocrine-gonadal axis to long daylengths and a decrease in circulating levels of gonadal steroids might result as a secondary consequence. Development of photorefractoriness in photoperiodic species has been viewed as physiological adaptation to pave way for rearing young ones and renew old and/or damaged feathers before start of the lean season and depletion of food resources in the habitat (Dawson, 2008).

In the present study, moult in PFs and BFs were accomplished in the middle of November taking about 135 days. Further, except that PFs moult in captive birds was delayed by a month as compared to wild birds, no sex related differences in the time and speed of moults were noticed in wild and captive groups. These results when compared with 110 days of PFs moult in red-vented bulbul (Dhondt, 1977), 112 days of PFs moult in yellow-vented bulbul (Ward,1969) and 126 days of PFs moult in grey-eyed bulbul (Pierce, 2009), appear to take longer duration for completion. Further, in stripe-throated bulbul, *Pycnonotus finlaysoni* wing- primaries renewal was accomplished within 136 days in males and 118 days in females (Kamtaeja *et al*, 2015). Simultaneous moult in PFs and BFs from June to November has also been reported in the subtropical tree sparrow, *Passer montanus* (Dixit and Singh, 2011). In the present study, slow pace of moulting in the red-vented bulbuls with no obvious differences in the pattern and duration of PFs and BFs renewal in male and female birds might account for sexes being equally involved in sharing parental responsibilities. High predation pressure owing to the presence of hawks, owls, black drongo, shrikes, swallows, crows, common myna *etc* keeps sexes alert and thus continue maintaining flight efficiency even during the periods of active moult to help young to grow and be able to nourish and defend themselves. Differences in the timings and duration of moult across species may be shaped by environmental constraints (Barta *et al*, 2008). Significantly, wing and body feather moults coincided with the surfeit abundance of insects and their larvae in the habitat which formed the most preferred food choice of bulbuls to support calorie requirements for refreshing and/or restructuring the body components. The strength of this suggestion may be relied in the fact that moulting process in bulbuls is often intercepted by gusty winds, incessant rains, hail storms and high predation pressure amidst heavy parental responsibilities.

Reproductive Behaviour

***Courtship display, Pair-bonding and Nest-site Selection*:** Courtship behaviour in red-vented bulbuls triggered at the earliest during the 2nd week of February to induce settlement of pair-bonding and dispersal of pairs in search of suitable nesting sites. Since behavioural attributes like mutual interactions, change in frequency and amplitude of chirping calls (Kumar, 2004, Neelakshi, unpublished) were noticed to commence concomitant with development of gonads, it may be assumed that these behavioural attributes had geared up under the influence of increasing milieux of sex steroids. Gonadal steroids are reported to influence seasonal changes in behavioural attributes of birds (Adkins-Regan, 1981; Crews, 1984; Wingfield and Soma, 2002; Balthazart *et al*, 2004). Lack of an expression of reproductive behaviour in birds with small gonads under non-stimulatory daylengths, supports this view (Neelakshi, unpublished, also see Chaper-III). Interestingly, well organized courtship displays and pair-bonding were observed towards the end of February culminated into nest-building and egg-laying during March/April in a small number of birds only. It may therefore, be argued that experienced breeders in native populations with relatively lower critical threshold of daylengths for gonadal growth, might have entered into nesting at the beginning of the breeding season. This statement relies on the fact that in some avian species, experienced individuals enter into breeding earlier than others in the population (Lewis *et al*, 2009; Lei *et al*, 2016). Such an adaptation may benefit birds in capitalizing maximum advantage on food resources available in the habitat with less intense vying. This supposition merits attention for reasons that sudden influx of nomadic populations of red-vented bulbuls in the study area occurred only during March/April. Further, in non-resident populations of bulbuls, pair-bonding could be accomplished within a week time and birds had immediately moved to occupy discrete pockets of the habitat keeping pace with resident populations and a sizable chunk of them entered into nesting during May/June along with native birds to constitute the peak breeding season. Yet again, the leftover birds had entered into nesting during August/September. This might include less experienced birds entering into breeding for the first time or the pairs which had accidently lost their nest and young ones earlier during the breeding season. There are reports to show that in many species, nestlings born late in the season are mandatorily required to pass through longer days for full expression of their neuroendocrine-gonadal axis (Nicholls *et al*, 1988) and that birds missing their clutches due to predation or inclement weather conditions, attempt re-nesting in the later part of the breeding season (Wingfield, 1983; Wingfield and Farner, 1993).

The observation that bulbul pairs visited trees one after the other and kept hopping from one branch to the other before they could mutually settle with a site suitable for fixing nest, might denote the extent of care birds take prior to stepping into the breeding process, probably to ensure successful rearing of the clutch and thus the better survival prospects of the species. Though, at the present, there is no convincing data to support that red-vented bulbuls might exhibit habitat and site tenacity but our long-term observations provide sufficient clue to take a positive note on it. The fact that bulbuls preferred fixing fresh nests in trees having nest-remains from the preceding breeding season corroborates the practice of natal philopatry. Side fidelity has also been reported in red-winged blackbirds (Beletsky and Orians, 1991), piping plovers (Friedrich *et al*, 2015) and common tailbirds (Feng *et al*, 2019).

Establishment of Territory: Soon after nesting site was determined, both the sexes of *Pycnonotus cafer stanfordi* were noticed to work out their territory consisting of approximately 30^2m area surrounding the spot within which the pair intended to prevent the entry of con-specific and/or hetero-specific individuals. A dry vertical branch on the tree-top was usually engaged as the view point by the male bird to guard its territory. This tendency was seen growing stronger with the furthering of breeding phases, such as nest construction and egg deposition, incubation and hatching. Territory establishment and inherent tendency to guard their nest and chicks have also been reported in stonechats (Marasco *et al*, 2011), songbirds (Goymann and Landys, 2011), greater rhea (Valdez *et al*, 2014), humming bird (González-Gómez *et al*, 2014), zebra finches (Prior *et al*, 2016; Pikus *et al*, 2017) and nuthatches (Randler and Randler, 2020). Interestingly, red-vented bulbuls were found to be well accustomed with the routine patrolling of their territory especially during non-breeding season as bulbuls could be seen regularly checking the extremities of their territory before dusk and chase away intruders like common myna, *Acidotheres tristis,* jungle crow, *Corvus macrorhynchos,* black drongo, *Dicrurus macrocercus,* and brown shrike, *Lanius cristatus etc.* This probably reflects at the inherent tendency of red-vented bulbuls to extend their territory owing to the expansion of their population dynamics. At the community level, red-vented bulbuls have been rated to figure in the top 3 most aggressive invasive birds on earth and has succeeded in establishing itself in 37 continental locations beyond its native ranges in the Asian zone (Brochier *et al*, 2010; Thibault *et al*, 2018a,b, 2019). However, during spring and summer months, as observed in chestnut-sided warblers (Byers, 2017), in bulbuls territorial integrity was negotiated to match the requirements of individual pairs. This feature has provided fair opportunity to many species to sneak into the area previously under the dominance of red-vented bulbuls. Many of them were found synchronizing their breeding timings with red-vented bulbuls and often noticed predating on their eggs and young ones (Neelakshi, unpublished). It is intriguing to seek explanation for the probable regulatory mechanisms for off-season and on-season expressions of territorial integrity in bulbuls. Though, it is yet to be established for red-vented bulbuls, seasonal changes in plasma levels of gonadal steroids like dihydroepiandrosterone (DHEA), progesterone, testosterone and their functional receptors have been reported to sustain territorial integrity of birds (Wingfield and Soma, 2002; Balthazart *et al*, 2004; Canoine *et al*, 2006; Pradhan *et al*, 2010; Marasco *et al*, 2011; Goymann and Landys, 2011; Rosvall, *et al*, 2012; Tetel and Acharya, 2013; Wingfield *et al*, 2018; Watts, 2020).

The Nest: Caliology of red-vented bulbuls during the past 5 years showed that red-vented bulbuls are altricial birds and habituated to build cup shaped nests generally at the centre of a multi-forked bough of the host-plant/tree, using locally available plant materials at an approximate height of 2 to 6 metres or more from the ground. The nest is always placed lurking amongst deep foliage at the centre of the tree probably to reduce vulnerability of the eggs and the young ones against predation. Additionally, it might add to the strategy that helps mitigate impact of probable injury inflicted by splashing rains, sleets and hails frequently sojourning in the study area during the breeding season. The height of nests off the ground was more at locations where vegetation under the host tree was relatively thick (Neelakshi, unpublished). This might reflect at the elementary strategy to protect fledglings against probable injury on their prematurely hoping out of the nest. In our study, no nest could be seen fixed on any human raised structures or at sites other than trees, shrubs and bushes as reported in some earlier studies (Inglis, 1922; Dixit, 1963; Lamba, 1976; Nanjappa, 1989; Sivasubramanian and Sunderamoorthy, 1992; Urfi and Keshubha, 1998). Likewise, nesting materials and the host plants preference have also been shown to vary depending on their availability and access in a particular habitat (Prabhakarachari *et al*, 1990; Rao *et al*, 2013; Zia *et al*, 2013; Awais *et al*, 2014; Zohaib *et al*, 2021). In the present case, 300 active nests were examined over a period of 5 years, during March to September, of which, 150 (50%) were fixed in the mango tree (*Mangifera indica,* March-September), 90 (30%) in wild cherry (*Prunus avium*, May - June), 25 (8.33%) in gulmohar (*Delonix regia,* June-August), 20 (6.67%) in pride of India plant species, (*Lagerstroemia speciosa,* May-June), 12 (4%) in bitter bean, stink bean or twisted cluster bean (*Parkia speciosa,* June-July), and 3 (1%) in marigold or Mexican sunflower plants (*Tithonia diversifolia*, June-July). It is interesting to note that preference for the host-plants for placing nests chiefly relied on the foliage and probable interference by con-specific and/or hetero-specific individuals. Mango trees perfectly fitted into such a requirement, for reasons that it is abundantly available in the study area and that it is densely packed with green leaves throughout the length of the breeding season. Secondly, owing to the uncontrollable damage of mango crops by mango stone weevil, *Sternochetus mangiferae*, which completes its life cycle inside bulbs and seeds, and adults emerge making holes in the fleshy mesocarp of ripening fruits, it hardly catches human attention and interventions. Despite wide abundance and presence of dense foliage, cherry trees made next option because of its ripening fruits during March-April which attracts many feeder birds and thus, nesting in cherry tree before May is likely to risk the territorial integrity of the breeders.

Nest-construction strategy: To our knowledge, this is the first report which details nest-construction strategy in red-vented bulbuls. Earlier reports have described breeding performance of red-vented bulbuls under varied habitats with no clear mention of their nest-building strategy (Inglis, 1922; Dixit, 1963; Lamba, 1976; Nanjappa, 1989; Sivasubramanian and Sunderamoorthy, 1992; Urfi and Keshubha, 1998; Thibault *et al*, 2018a,b, 2019; Zohaib *et al*, 2021). Nest construction began with preparation of nest-rim and its subsequent sealing with the host-bough using compact masses of spider webs. Occasional use of jute and plastic threads to tie up nest-rim with the host-bough were also envisaged. No evidence of use of metal wires in nest-building as described by Lamba (1968) could be seen in the present case. Wounding and/ or corroding soft inner surfaces of the fork in some instances before fixing them with the rim represents unique feature of red-vented bulbuls, not described ever before for any other bird species. Care exercised by birds prior to the fixing of rim with the host-branch is undoubtedly an intelligent deed to prevent nest-dislodge by gusty winds not uncommon at the study sites during the breeding season. Leaf-rachis of Gulmohar (*Delonix regia),* Morning Glory (*Ipomoea* *hederifolia lutea*, *Ipomoea alba* and *Ipomoea indica*) vines were frequently used in making nest-rim. Inverted dome-shaped lattice was created using leaf-rachis of Gulmohar and culms of thatch grass by connecting their opposite sides with the rim and then seal them using spider webs. Following day, the lattice was slowly filled with loose interlacing of dry leaf blades and thin stems of thatch grass available at the study sites. For two consecutive days, enmeshed materials were compacted starting from the top towards the bottom. On day 4, the bottom of the nest was interwoven with thatch grass stems and long leaf blades. Finally, on day 4 or 5, the bottom was interlaced with thatch grass flowers and thin stems and was ready for use. On clear sunny days, a nest was completed within 5-6 days but when intercepted by inclement weather conditions, the finish was delayed owing to the want of dry nesting materials in the habitat. Similar duration for nest completion has recently been reported in red-vented bulbuls nesting at 30^{o}N (Zohaib *et al*, 2021). It is thus obvious that nest-architecture in bulbuls represents evolutionary traits of the species and within its normal bound, habitat ecology and nest building materials do not profoundly affect the timing of its completion. However, threat perception on account of anthropogenic activities, recurrent disturbances by predators amidst nest building, prompted birds to abandon nest unfinished owing to search for a newer site as reported in Syberian jay (Eggers *et al*, 2006). Further, the observation that no two active nests ever existed together in the same tree may be ascribed to acute territorial integrity of red-vented bulbuls pertinent during the breeding season as evidenced in the chestnut-sided warblers (Byers, 2017).

The observation that entry and exit routes to nest were determined at the time of placing them at the bough as the mates followed different routes to enter the nest and their egress. This feature of the red-vented bulbuls is unique and to our knowledge so far, this is not reported for any other avian species. Further, significantly less base area with significantly more depth of nests with the clutch size of 02 eggs as compared to the nest with 03 eggs may denote that clutch size in red-vented bulbuls is determined in relation to the basement area and the depth of the nest. This fact has been examined by a group of 84 scientists who had sampling from 17,472 nests for 21 species nesting at different latitudes (Moller, *et al,* 2014), and the outcome supports present findings in red-vented bulbuls. Mismatch between nest size and the clutch volume has been shown to reduce egg survival and fledgling success in black tailed gull (Lee and ChilYoo, 2016). It is however, intriguing to speculate whether clutch size is determined before shaping the nest or the size of the nest determines the presumptive clutch size? Logically, the former outweighs latter possibility for the reason that nest size is exclusively under the voluntary control of birds.

Mating: A mating pair of red-vented bulbul could be observed any day from March/April to September. But, the peak mating season clustered around May/June. Owing to the fact that mating in birds is influenced by increased circulating levels of gonadal steroids (Wingfield and Soma, 2002; Balthazart *et al*, 2004; Pradhan *et al*, 2010; Roswall, *et al*, 2012; Tetel and Acharya, 2013; Wingfield *et al*, 2018; Watts, 2020), it may be deduced that mating behaviour in the red-vented bulbuls is induced and/or maintained by gonadal hormones at critical threshold concentrations higher than the levels necessary for inducing courtship and territorial integrity. Mating was seen to occur generally when a pair was roosting after completing a short spell of nest construction. The fore plays included male's approaching the female who instantly leaped forward to entice him before she gave access to mount her. Enticement prior to the mating act has been reported in many birds and may be causally related to the copulation with elevated vigour to ensure transfer of maximum quantity of sperms in the female's genital tract (Lal *et al*, 2012; Watts, 2020). The observation that female gave access to the male to mount her only in inclined position, may be accounted to facilitate the transfer of seminal fluid in the oviduct. Further, since mating is viewed as the key step towards fertilization, in bulbuls likewise many other birds (Lal *et al*, 2007), the process was instantly repeated two to three times probably to maximize sperm storage by the female.

Egg Laying, Clutch Size and Incubation: Nidology of 300 nests from 2017-2021 revealed that active nests of red-vented bulbuls could be found in the study area between March/April-September. When viewed together with reports published on breeding performance of red-vented bulbuls at different latitudes within and outside the Indian subcontinent (McCann, 1932; Dutt, 1932; Lamba, 1968; Vijayan, 1980; Bhatt and Kumar, 2001; Balkrishnan, 2010; Prajapati *et al*, 2011; Rao *et al*, 2013; Awais *et al*, 2014; Thiabault *et al*, 2018, 2019, Zohaib *et al*, 2021), it may be argued that timing and length of breeding season may differ at different locations but common feature entails peak nesting season to coincide with lighting period above 12 hrs. This may imply that in *Pycnonotus cafer stanfordi* daylengths in excess of 12 hrs are necessary for the activation of neuroendocrine-gonadal axis to manifest final maturation of gonads, mating and shedding of viable gametes. The fact that testes of red-vented bulbuls developed under simulated daylengths in excess of 15-16 hrs/day and fixed durations of 8-9 hrs/day prevented testicular growth supports this view (Lal and Thapliyal, 1982a, see Chapter III). In a sample size of 150 nests studied during 2017- 2018, only 30 had a clutch size of 02 eggs and the remaining 120 nests had 03 eggs each with an average clutch size of 2.88±0.05. Eggs were deposited singly on consecutive days in the forenoon owing to completion of the clutch size within 2 or 3 days. Present results corroborate findings of other investigators on the clutch size in native populations of red-vented bulbuls and their congeners nesting in alien ranges where the species is presumed to have been transplanted from Asia (Watling, 1978; Lowe *et al*, 2000**;** Mazumdar and Kumar, 2007; Jetz *et al*, 2008; Thibault *et al*, 2018; Fishpool and Tobias, 2019). It is thus obvious that unlike reports available for some avian species (Lack, 1946 a,b; Soler and Soler, 1992; Sanz, 1999; Cooper *et al*, 2005; Jetz *et al*, 2008; Evans *et al*, 2009; Griebeler *et al*, 2010; Pienaar *et al*, 2013; Xiao *et al*, 2017), in the red-vented bulbuls, latitude and daylengths may not influence the clutch size. Lack of an effect of egg manipulation amidst laying on absolute egg-counts in the final clutch size suggests that red-vented bulbul is a determinate layer. Indeterminate layers respond to egg manipulations during the course of deposition and thus influence the final clutch size (Anderson, 1989; Briggs, 1991; Kennedy, 1991). Ovaries of free living and captive birds examined during the breeding season exhibited follicular hierarchy confined to 2-3 eggs only (Neelakshi, unpublished). This strongly advocates in favour of genetic determination of the clutch size. The size and mass of eggs observed in the present study, marginally exceeded values reported for these indices in the red-vented bulbuls nesting in other habitats (Rao *et al*, 2013; Sharma and Sharma, 2013; Awais *et al*, 2014). It is difficult to speculate whether differences account for differences in the subspecies status of red-vented bulbuls or it escalates combined with food availability in the habitat and/or nutritional status of the mates (Horak *et al*, 1995). Further, the observations that nests with only 02 eggs were found either at the start of the breeding season in March/April or towards the end of the nesting season in September. This may not simply be a coincidence but may narrate limitations at supply of quality food resources in the habitat and/or nutritional status of the mates as reported in some avian species (Dunn *et al*, 2000; Jetz *et al* 2008). The fact that insect fauna flourished in the study area during April/May - August and larvae of insects constituted predominant part of food for tender fledglings, lends support to this suggestion.

Nidology of 40 active nests showed that incubation commenced following deposition of penultimate or ultimate egg of the clutch. Hatching asynchrony was established in 69.56% cases, synchronized hatching occurred in 17.39% eggs and the remainder 13.05% eggs failed to hatch. Asynchronous and synchronous hatching has also been reported in other passerines (Fishpool and Tobias, 2019). In the present case mean incubation period was recorded at 14±0.11 days. Ali and Ripley (1971) have also described 14 days of incubation period in the red-vented bulbul. Recent reports published on breeding performance of red-vented bulbuls from different latitudes within its native range have however, variously described incubation periods ranging from 9-16 days (Prajapati *et al*, 2011; Sharma and Sharma, 2013; Rao *et al*, 2013; Zia *et al*, 2013; Awais *et al*, 2014; Zohaib *et al*, 2021). In the red-vented bulbul only female sex is reported to perform incubation and the male guards the nest against con-specific and/or hetero-specific intruders (Fishpool and Tobias, 2019). In a separate study incubating birds caught during night were laparotomized to ascertain sex and all were invariably found to be female (Neelakshi, unpublished). Incubation was continuous during night (~18.30 hr - 5.30 hr). However, during day time parents left the nest intermittently for foraging with hourly frequency at 2.70±0.81. On the average female spent 63±15% of natural light hours on incubating. An average on-bout and off-bout timings were at 16.54±7.32 min and 11.51±6.10 min respectively. In the present study, red-vented bulbuls were found to spend less time on incubation compared with some passerine species as *Anthus pratensis* (77.19%) (Kovank *et al*, 2009), *Junco phaeonotus* (76.9%) (Weathers and Sullivan, 1989) and at par with *Dendroica caerulescens* (64%) (Joyce *et al* 2001) and *Pycnonotus jocosus* (58±13%) (Li *et al*, 2015). Day time incubation with short spells of interruptions may be accounted to the moderate ambient temperature which fluctuated within a narrow range of 28.1^{oC}-34.1^{oC} between 5.30-18.5 hr at the study site. In chicken temperature below 26^{oC} is reported to suspend development of embryos (Conway and Martin, 2001). It may therefore be inferred that moderate ambient temperature in the study area would provide fair foraging opportunity to off-bout mates without risking the embryonic development.

Hatching and Parental Care: Hatching of eggs occurred one by one at a gap of 24-30 hours. Newly born hatchlings were naked, their eyes were closed but acoustic perceptions were fully functional. Obviously, like many other passerines (Anderson, 1989; Kennedy, 1991), red-vented bulbuls are nidicolous or altricial birds in which young ones demand prolonged nurture and care to complete their developmental stages. In the present case, female parent continued keeping hatchlings warm and both the sexes were seen guarding them against con-specific and hetero-specific intruders/predators. This feature was noticed commencing concurrent to the presence of first egg in the nest. During egg deposition, parents were seen sounding alarms following an intruder or a predator having access to their territory. This tendency grew further with next egg added to the nest. Towards completion of the clutch size, birds became extra vigilant and showed inherent tendency to resist upsets resulting due to anthropogenic activities or the presence of predators and/or intruders in their territory. Parental care continued increasing with successive increase in clutch size and heightened with first hatchling appearing in the nest to pose obligatory feeding requirements within an hour. Both the sexes were observed fetching soft morsel of food comprising chiefly of larvae of moths and butterflies, insects (grass hoppers, ants and winged termites *etc*), spiders, small flower buds and fruits (banana, papaya, cherry, mulberry, black plums, black nightshade and velvetleaf fruits *etc*) to nourish young ones. It was interesting to note that nestlings were made accustomed to swallow larger pieces of food before their final reward. This may add to the adaptive value as it helps avoiding choking of throat during feeding. Territorial integrity and parental care were seen operating at the extreme with nestlings reaching fledging stage when birds were suddenly overwhelmed with responsibilities of nurture and safety concerns so long young birds were skilled to fetch food and master tricks to evade predation. This characteristic of red-vented bulbuls has much similarity with other passerines (Cockburn, 2006) which exhibit biparental care of the young ones.

Development of Chicks: To our knowledge this is the first report to describe developmental stages of the *Pycnonotus cafer stanfordi*. Other reports made from different habitats are based on casual records on nest and nestlings and thus do not portray synchronized development of hatchlings (Mummigatti *et al*, 2001; Zohaib *et al*, 2021). Feather papillae appeared on 4th day, grew further through days 5-7. Eyes opened on 8th day. As the eyes opened, young ones were noticed exhibiting fear complex. At this stage, nestlings began moving their wings and making sounds louder than before. Female continued spending night in the nest along with nestlings. Between days 9-13 body of nestlings was covered with feathers and by the day 12, they began fluttering wings and became restless to escape from the nest. It is interesting to note that parent birds intended to synchronize the development of chicks by strategically nourishing them, but the youngest nestling was often found leaving nest delayed by a day or two. Breeding success (hatching success, 86.9% and nest success, 60%) in the present case was very high as compared to reports available for red-vented bulbuls from other ecosystems from within its native ranges (Mummigatti *et al*, 2001; Prajapati *et al*, 2011; Sharma and Sharma, 2013; Rao *et al*, 2013; Zia *et al*, 2013; Awais *et al*, 2014; Zohaib *et al*, 2021). High breeding success in populations of red-vented bulbuls in Zunheboto district might account for good forest cover with surplus availability of quality food resources and relatively lesser degree of predation pressure. As such no other avian species in place could effectively match the extension and expansion strategies of red-vented bulbuls. The observation that fledging occurred by day 14, corroborates findings of many other workers (Ali and Ripley, 1971; Mummigatti *et al*, 2001; Prajapati *et al*, 2011; Rao *et al*, 2013; Sharma and Sharma, 2013; Zia *et al*, 2013; Awais *et al*, 2014).

Skilling the Young: Post-fledging scenario reflected at the most prudent strategy which culminated into skilling chicks in procuring nourishment to fine tune their survival strategy. Parents escorted fledglings to the adjoining space with relatively less frequent human interference. Such areas were generally under the moderate grass cover with less prominent bushes growing around. Birds nesting around this place indiscriminately used this land mass for collecting nourishment and skilling their young who could hardly hop for few yards. Parents resorted to train chicks daily for about an hour, preferably at dawn and dusk, to instil characteristics essential for enriching survival prospects of the species. The process clicked with catching of insects by parents to meet obligatory feeding requirements of the young ones. The move sparked instinctive traits in the young who hustled to grab insects to nourish them. After about half an hour, parents were noticed guiding young ones to hop in the bush and leaving them there, they moved around for foraging. Interestingly, all along, parents and young remained at acoustic linkages and very often one of the parents visited them with a morsel of food. Sensing the presence of predators around, parents alarmed young to hide from the sight of predators. Most amazingly, chicks responded by instantly pushing them to a corner and sit ideal with their heads buried under feathers until parents arrived to escort them. Training schedule was noticed to continue for about two weeks to enable young birds become fully equipped with survival skills in the contemporary ecosystem. During night, parents and young roosted together in bushy trees until such time they joined a larger foraging flock. Skilling of young bulbuls is unique feature, and to our knowledge, this phenomenon has not been described for any other avian species. The characteristic traits might denote necessary trade-off enhancing survival instincts of the species under varied habitats. When viewed together with aggressive nature and extension ability towards population expansion in its native and alien ranges, it seems logical to surmise that skilling would synergise survival fitness of red-vented bulbuls, a species immensely endowed with inherent adaptive abilities.

Murmuration Display: Present observations reveal that bulbul murmurations occur during post-breeding season when smaller groups of birds assembled to forage in discrete pockets of the habitat. During autumn months (August to October), most of the foraging sites were flourished with insect fauna with abundant availability of grass hoppers, winged termites and larvae of moths which constitute preferred food resources of both, red-vented and black-headed bulbuls (Neelakshi, unpublished). Besides, other insect species and a large variety of fruits and flowers available in the habitat constituted potential sources of organic food stuffs and minerals to help foster growth of young ones and to replenish energy stocks of parents exhausted during intense breeding activities and feather renewal. Further, since food resources in the habitat are unevenly scattered, birds continued getting amassed to forage and share a common roost. At Lumami, during late autumn months, it is not uncommon to see 100-200 red-vented and black-headed bulbuls congregating at common roosting site, generally in trees on the hill top, immediately after foraging in the morning, day time and/or at dusk. However, a flocking behaviour manifesting into massive murmuration involving several hundred (>1000) birds during morning hours as witnessed first on 12th October 2019 was an unusual occurrence, not seen before, on or near the university campus. Owing to the fact that unlike starlings, bulbul flocks flew and swooped closer to their roost, it is likely that such episodes occur regularly during autumn months at one or other sites, but due to presence of high hills around, this may not become visible from the university campus. This possibility was explored by raising citizen's query involving people living in the hills surrounding university campus and nearby villages, but all ended into utter dismay. To our knowledge, so far, there is no report of murmuration in bulbul species from their native and/or alien locations.

Murmurations are strikingly unique behavioural patterns which multiple individuals of a species and sometimes, from across species, express together for the safety against common predators (Sumpter, 2006). When looked from wider perspectives, murmurations are to benefit birds on three accounts (King, 2012): First, 'The Warmer Together' hypothesis has been proposed to explain murmurations during late autumn and winter months immediately before roosting, for reasons, that it would keep the roost warm with additional advantage that birds would find better opportunity for food resources, once the roost disperses. Such a possibility has been tested in socially roosting red-billed quelea, *Quelea quelea* and cliff swallows, *Hirundo pyrrhonota* (de Groot, 1980; Brown, 1986) and could be important in cold weather (Elgar, 1986). However, no convincing data are available to support this hypothesis except for starlings which prefer larger roosts (Davis and Lussenbop, 1970) and wren, *Troglodyte troglodytes*, which vocally advertise communal roosts in colder weather (Armstrong, 1955). Second, the 'Dilution Effect' states that the size of flock in a murmuration is negatively correlated with the individual predation risk (Hamilton, 1971; Olson *et al*, 2016). Its mutual bearing on 'Detection Effect' and 'Predator Confusion Effects' seemingly serve in much better ways to protect individual member in a flying squad (Powell, 1974; Lazarus, 1979; Pulliam *et al*, 1982; Elgar, 1989; Beauchamp, 2008). This with 'Safer Together Hypothesis' operates in taxonomically and ecologically diverse animal groups to maximize their survival prospects. Fish schooling to distract predators (Krause and Godin, 1994; Hemelrijk and Hildenbrandt, 2012), feeding of ants using pheromone trails (Hart and Jackson, 2006), herds of migrating blue wildebeest (Thirgood *et al*, 2004) and pre-roosting aerial exercises of birds (Goodenough *et al*, 2017), are few examples to cite. In birds, murmuration displays have been noticed in many species, *viz*. black birds, starlings, shorebirds, swallows, robins, flamingos, cranes and pigeons (Apthorp, 2017; Rao, 2018) during late autumn and winter months, immediately before settling at roosts. Of all the avian murmurations described so far, displays in the European starlings have gained relatively wider popularity, for reasons, that it displays huge mesmerizing mobbing flocks at dusk which sometimes, include birds exceeding 120,000 in number prior to funnelling down to their roost (Young *et al*, 2013; Goodenough *et al*, 2017).

It is intriguing to note that existing literature add to resolve issues related to culmination and possible causes of murmuration, but no clues on how it clicks? Present finding pioneers this fact in joint exercise by red-vented and polymorphic forms of black-headed bulbuls wherein murmurations were noticed to spark and sustained by identified roost leadership. It however, remains to establish whether red-vented or black-headed bulbuls had triggered the process. However, owing to participation by larger numbers, black-headed bulbuls appear to be in the driving seat. Interestingly, common roosting sites were not inflexible and kept shifting as per need. Further, murmuration displays particularly during morning hours might denote definite strategy to cope with predation pressure from resident raptors like Hawks, *Accipiter badius, Accipiter nisus*, (ZSI, 2006), and migratory Amur Falcon, *Falco amurensis* roosting in the study area in route to their breeding grounds in the south-African plateau (Bildstein, 2006). Further, Amur falcons are reported to selectively forage on locusts, grass-hoppers, winged termites, passerines, amphibians and small mammals (Pietersen and Symes, 2010). Since, grass-hoppers, locusts and termites also form favourite food of bulbuls during this period, scarcity of quality food resources in the habitat might necessitate search for newer potential feeding sites and transfer of information may occur by way of murmuration as proposed for starlings, *Sturnus vulgaris* (Attanasi *et al*, 2014).

It is interesting to note that bulbul murmurations manifest during the 2nd week of October coinciding arrival of Amur Falcons in the area. It is noteworthy that owing to the presence of Falcon flocks hovering over, murmurations either did not escalate or were terminated intermittently. Further, bulbuls might avoid murmurations at dusk to maintain secrecy about location of their roosts which are not inflexible probably to cope with predation by foraging flocks of Amur falcons. In addition, murmurations in bulbuls may be viewed as a rehearsal for autumnal migration. Seemingly, it sounds strange as both, red-vented and black-headed bulbuls are considered as truly residential. Such a supposition stems from the recent report that major chunk of *Pycnonotus cafer stanfordi* populations breeding near Bhamo village area of Myanmar leave the site after breeding season is over (Islam and Williams, 2020). Partial migration is also reported in some other residential forms (Hegemann *et al*, 2019). Further, the fact that all bulbul populations at Lumami did not participate in the murmuration exercise and that every year, during October to March period, drastic decline in population dynamics of red-vented and black-headed bulbuls occur in the study area, strengthens this view (Neelakshi, unpublished). It may therefore, be surmised that bulbul populations swarming up in the study area during March/April are basically the non-resident forms which visit this part of the state for raising progeny and once breeding is over and the youngs are grown to care for themselves, they depart from the place probably to winter at lower latitudes (Islam and Williams, 2020). A Joint murmuration by Red-vented and black-headed bulbuls may add to the cause of ‘safer together’ especially in the years of high prevalence of Amur falcons in the study area.

Table - 1: Seasonal changes in Animal and Plant foods contents in Gut of wild Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami (26°N).*

Months *2017*	Total Gut content (*g±SE*)	Plant Food content *(g±SE)*	Animal Food Content *(g±SE)*	Plant Food content *(g%±SE)*	Animal Food content *(g%±SE)*
January	5.26±0.15	3.93±0.17	1.33±0.14[e]	74.70±2.47	25.30±2.47
February	5.28±1.37	4.23±0.32	1.03±0.05[f]	79.14±1.69	20.86±1.69
March	4.68±0.19	3.60±0.19	1.08±0.23[d]	77.36±4.60	22.64±4.60
April	4.83±0.09	3.68±0.16	1.15±0.16[d]	76.21±3.38	23.79±3.38
May	4.47±0.15	3.25±0.21	1.22±0.22[d]	72.82±4.68	27.18±4.68
June	4.50±0.11	2.90±0.17[b]	1.60±0.23	64.63±4.52	35.37±4.52
July	4.28±0.18	2.50±0.24[c]	1.78±0.17	58.29±6.15	41.71±6.15
August	4.58±0.09	2.60±0.15[c]	1.98±0.08	56.73±2.30	43.27±2.30
September	4.98±0.09	3.30±0.06[a]	1.68±0.10	66.34±0.63	33.66±0.63
October	5.20±0.14	3.55±0.21	1.85±0.35	65.38±2.98	34.62±2.98
November	4.98±0.24	3.75±1.17	1.23±0.28[d]	74.43±5.71	24.58±5.71
December	4.98±0.09	3.90±0.27	1.03±0.26[d]	78.38±4.93	21.65±4.98

*Values are Mean±*SE*. n=4 in each group.Total gut contents across months, $F_{(11,36)}$ =1.76 (ANOVA). Plant food in Gut-contents across months, $F_{(11,36)}$ = 6.22, $p<0.0001$ (ANOVA).Animal food in gut-contents across months, $F_{(11,36)}$ = 3.31, $p<0.001$(ANOVA)

[a,b,c] differ from the value of February at $p<0.05$, <0.01 and <0.001 level respectively (Student's '*t*' test).

[d,e,f] differ from August value at $p<0.05$, <0.01 and <0.001 respectively (Student's '*t*' test).

Table-2 : Seasonal changes in Body Mass, Testicular Size and Moult Score of Captive Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan maintained under natural lighting (NDL) at Lumami (26°N)*

Months *2017*	Year	No. of birds	Body Mass (*g±SE*)	Testis Size ($mm^3 \pm SE$)	Primary Feather Score	Body Feather Score
January	2017	06	36.95±0.43	0.85±0.08[c]	-	-
February	2017	06	37.37±0.48	0.87±0.51[c]	-	-
March	2017	06	36.95±0.46	20.53±1.88[c]	-	-
April	2017	06	37.33±0.42	66.89±12.15[b]	-	-
May	2017	06	37.75±0.47	125.79±13.35	-	-
June	2017	06	36.55±0.45	84.34±9.47[a]	4.67±0.49	2.83±0.33[h]
July	2017	06	35.67±0.47[a]	47.26±5.80[c]	9.33±.61	5.30±0.47
August	2017	06	35.38±0.34[a]	12.89±2.72[c]	21.67±2.03[d]	6.27±0.61
September	2017	06	35.68±0.46[a]	6.08±0.20[c]	30.00±1.27	4.33±0.65[g]
October	2017	06	38.20±0.24	3.54±1.04[c]	17.33±3.5[d]	2.42±0.40[h]
November	2017	06	38.15±0.26	0.78±0.08[c]	3.00±0.37[f]	1.42±0.23[i]
December	2017	06	37.80±0.28	0.63±0.07[c]	-	-

*****Values are Mean±*SE*; Body Mass: F($_{11,60}$)=5.44, p<0.001; Testicular Volume: F($_{11,60}$)=32.92, p<0.0001 (based on One way Independent Measure of ANOVA); Primary Feather Moult Score: F($_{5,30}$)=36,26, p<0.0001; Body Feather Moult Score: F($_{5,30}$)=12.07, p<0.0001 (One way ANOVA).

[a,b,c] differ from value of May month at p<0.05, <0.01 and <0.001 respectively (Student's '*t*' test).

[d,f] differ from the value of PF Score in September at p<0.05 and <0.001 respectively (Student's '*t*' test).

[g,h,i] differ from the value of BF Score in August at p<0.05, p<0.01and <0.001 respectively (Student's '*t*' test).

Table-3: Seasonal changes in Body Mass and Follicular Size of Captive Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan maintained under natural lighting at Lumami (26^{o}N).*

Months *2017*	Year	No. of birds	Body Mass *(g±SE)*	Follicle Size *(mm±SE)*	Primary Feather Score	Body Feather Score
January	2017	06	36.40±0.73	0.50±0.00^c	-	-
February	2017	06	37.38±0.48	0.67±0.11^c	-	-
March	2017	06	37.71±0.36	1.0±0.16^c	-	-
April	2017	06	37.87±0.21	1.40±0.19^c	-	-
May	2017	06	38.22±0.29	2.63±0.11	-	-
June	2017	06	36.77±0.36^a	2.70±0.14	3.00±0.53^c	3.67±0.76^f
July	2017	06	36.07±0.47^a	1.60±0.19^c	10.42±1.20^c	6.42±0.64
August	2017	06	36.88±0.29^a	1.25±0.11^c	19.00±1.77^c	7.25±0.44
September	2017	06	36.97±0.45^a	0.60±0.10^c	31.72±0.89	6.33±0.67
October	2017	06	38.40±0.46	0.50±0.00^c	17.88±1.65^c	4.50±0.50^e
November	2017	06	38.50±0.36	0.25±0.00^c	2.00±0.52^c	1.75±0.31^f
December	2017	06	38.45±0.10	0.25±0.00^c	-	-

*****Values are Mean ± *SEM*, n=6 in each group; Body Mass: F($_{11,60}$)=3.04, $p<0.005$; Follicular Size: F($_{11,60}$)=118, $p<0.0001$ (One way Independent Measure of ANOVA); Primary feather Moult Score: F($_{5,30}$)=87.77, $p<0.0001$; Body Feather Moult Score: F($_{5,30}$)=12.68, $p<0.0001$ (One way Independent Measure of ANOVA).

^{a,b,c} differ from within group value of May for different indices at $p<0.05$, $p<0.01$ and $p<0.001$ respectively.

^{c} differs from the value of PF Score in September at $p<0.0001$ (Student's '*t*' test).

^{e,f} differ from the value of BF Score in August at $p<0.05$ and $p<0.001$ respectively (Student's '*t*' test).

Table-4 : Seasonal changes in Body Mass, Testicular Size, Weight, Vasdeferens Weight and Moult Score of wild Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami (26^{o}N).*

Months *2017*	Body Mass *(g±SE)*	Testis Size *(mm^{3}±SE)*	Paired Testis Mass *(mg±SE)*	Vasdeferens Mass *(mg±SE)*	Primary Feather Score -	Body Feather Score
January	37.42±0.76^{a}	1.23±0.38^{c}	1.90±0.79^{c}	2.10±23^{c}	-	-
February	37.87±0.67	3.80±1.07^{c}	3.06±1.25^{c}	2.32±0.48^{c}	-	-
March	37.57±0.70	24.05±6.08^{c}	7.78±0.96^{c}	5.38±0.41^{c}	-	-
April	37.78±0.62	142.48±11.46^{b}	125.93±7.08^{e}	11.71±1.45^{e}	-	-
May	39.38±0.36	216.26±11.12	224.95±31.45	20.50±1.07	-	-
June	36.68±0.38^{a}	84.25±13.46^{c}	81.51±25.25^{c}	14.30±1.13^{c}	6.83±0.87^{f}	2.13±0.43^{e}
July	37.02±0.64^{a}	29.48±7.03^{c}	12.88±2.69^{c}	7.95±1.09^{c}	20.00±.1.46^{e}	5.55±0.64
August	37.53±0.46^{a}	6.50±2.60^{c}	4.04±1.02^{c}	4.12±0.55^{c}	29.33±1.22	7.50±0.56
September	37.80±0.49^{a}	2.83±0.81^{c}	2.42±0.71^{c}	3.73±0.60^{c}	25.33±1.23^{d}	4.17±0.79^{d}
October	38.27±0.49	1.84±0.33^{c}	2.48±0.31^{c}	3.49±0.29^{c}	8.50±0.62^{f}	2.42±0.46^{e}
November	38.15±0.26	0.71±0.12^{c}	2.19±0.22^{c}	2.87±0.41^{c}	1.83±0.40^{f}	1.08±0.19^{f}
December	37.80±0.28	0.68±0.11^{c}	2,20±0.18^{c}	2.80±0.15^{c}	-	-

*****Values are Mean ± *SEM*, n=6 in each group. Body Mass: F($_{11,60}$)=2.22, p<0.005; Testicular Volume: F($_{11,60}$)=116.98, p<0.0001; Paired Testis Mass: F($_{11,60}$) =71.56, p<0.0001: Vasdeferens Mass: F($_{11,60}$)= 90.55 (ANOVA).
Primary Feather Moult Score: F($_{5,30}$)=192.35, p<0.0001; Body Feather Moult Score: F($_{5,30}$)=19.86, p<0.0001 (ANOVA).
^{a,b,c} differ from value of May for indices within parameters at p<0.05, <0.01 and <0.001 respectively (Student's '*t*' test).
^{d,e,f} differ from PF and BF Scores compared with August value at p<0.05,p<0.01 and p<0.001 respectively (Student's '*t*' test).

Table-5 : Seasonal Changes in Body Mass, Follicular Size, Ovarian Mass, Oviduct Mass and Moult Score of wild Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami (26^{o}N).*

Months *2017*	Body Mass *(g±SE)*	Follicle Size *(mm±SE)*	Ovarian Mass *(mg±SE)*	Oviduct Mass *(mg±SE)*	Primary Feather Score	Body Feather Score
January	37.93±0.43	0.85±0.28^{c}	10.15±0.88^{c}	6.98±0.42^{c}	-	-
February	37.45±0.58	1.50±0.20^{c}	19.20±2.61^{c}	7.09±2.53^{c}	-	-
March	37.58±0.46	2.54±0.16^{c}	29.09±1.70^{c}	11.00±0.81^{c}	-	-
April	37.88±0.62	2.67±0.17^{b}	36.49±2.94^{c}	15.98±1.64^{c}	-	-
May	39.57±0.57	3.67±0.12	78.68±3.02	25.58±1.13	-	-
June	37.55±0.45^{a}	3.08±0.24^{a}	43.69±5.89^{c}	19.73±0.79^{c}	9.17±0.83	3.17±0.48^{f}
July	36.87±0.57^{b}	1.75±0.21^{b}	20.22±2.77^{c}	12.94±0.92^{c}	21.00±.1.23^{f}	4.66±0.45^{f}
August	36.72±0.54^{b}	1.58±0.24^{c}	17.23±3.18^{c}	12.24±1.32^{c}	31.00±1.00	8.33±0.21
September	36.27±0.56^{b}	1.50±0.18^{c}	16.21±2.53^{c}	11.73±0.84^{c}	20.00±1.15^{f}	4.83±0.45^{f}
October	38.62±0.44	1.42±0.20^{c}	12.92±2.55^{c}	9.64±1,13^{c}	8.33±0.95^{f}	2.33±0.61^{f}
November	38.52±0.56	1.33±0.17^{c}	13.27±2.33^{c}	8.75±0.89^{c}	1.92±0.38^{f}	1.50±0.34^{f}
December	37.18±0.58	0.65±0.25^{c}	9.18±0.67^{c}	7.29±0.37^{c}	-	-

*****Values are Mean ± SEM, n=6 in each group. Body Mass: F($_{11,60}$)=3.08, p<0.001; Follicular Size: F($_{11,60}$)=33.74, p<0.0001; Ovarian Mass: F($_{11,60}$)=50.11, p<0.0001; Oviduct Mass: F($_{11,60}$)=56.32, p<0.0001 (ANOVA).
Primary feather Moult Score: F($_{5,30}$)=69.71, p<0.0001; Body Feather Moult Score: F($_{5,30}$)=26.17, p<0.0001 (ANOVA).
^{a,b,c} differ from value of May within parameters at p<0.05, p<0.01 and p<0.001 respectively (Student's '*t*' test).
^{f} differs from the values of PF and BF Scores in August at p<0.001 (Student's '*t*' test).

Table-6: Diameter, Depth and Basement Area of the Nest of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami (26°N).

Nest Diameter at Rim Level (mm±SE)		Total Depth of Nest (mm±SE)		Basement Area of the Nest (mm^2±SE)	
With 3 Eggs (N=120)	With 2 Eggs (N=30)	With 3 Eggs (N=120)	With 2 Eggs (N=30)	With 3 Eggs (N=120)	With 2 Eggs (N=30)
67.62±0.83 *mm*	62.83±0.47 *mm*[b]	37.39±0.22 *mm*	42.40±0.46 *mm*[c]	150.34±3.15	117.80±2.10[c]

[b,c] differ from the corresponding values of parameters as compared to nest having 3 eggs as the clutch size at $p<0.05$ and $p<0.001$ levels respectively (Student's 't' test).

Table 7**:** Influence of egg manipulation during deposition on clutch size of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami (26^{o}N).

Date of Egg removal (n**=6)**	Eggs in the nest at removal	No. of Eggs removed	No.of Eggs laid after removal	Factual Clutch size
15.05.2019-20.06.2019	02	01	01	03
17.05.2019-20.06.2019	03	01	0	03
Date of egg addition (n=6)	Eggs in the nest at addition	No. of Eggs added	Eggs laid after addition	Factual Clutch size
10.05.2019-16.06.2019	01	01	02	04 (3+1)
22.05.2019-16.06.2019	02	01	01	04 (3+1)

Table 8: Mean Size and Mass of Eggs of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami (26°N).*

Egg Length (*mm*) ± SE	Egg Width (*mm*) ± SE	Egg Volume (*mm*^{3}) ± SE	Egg Mass (*g*) ± SE
22.80 ± 0.09 (Range, 21-24) n=115	16.75 ± 0.09 (Range, 15-18) n=115	3347.02 ± 65.71 n=115	3.42 ±0.08 (Range, 3.0-3.8) n=115

*Values are Mean±SEM

Table 9: Breeding Success in Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami (26°N) during 2017.

No. of eggs (laid)	No. of eggs (hatched)	No. of eggs (unhatched)	Hatching (%)	Fledging (%)
115	100	15	86.9	60.0

CHAPTER – III

Seasonal Changes in Sensitivity of the Photoperiodic Response System in Red-Vented Bulbul, *Pycnonotus cafer stanfordi* Deignan

INTRODUCTION

In birds, likewise mammals (Bradshaw *et al,* 2007; Kriegsfeld and Bittman, 2010; Walton *et al*, 2011; Nakane and Yoshimura, 2019), seasonality in physiological and behavioural attributes relies on a complex interplay between multiple aspects of physical world and the individuals' internal milieux. Environmental cues such as light, temperature, humidity, rainfall and change in landscape *etc* alone or in combination trigger neuroendocrine events to effect temporal adjustments between proximate factors and the internal physiology to precipitate energetically expensive events like migration, moult and reproduction temporally spaced and tied to the availability of food resources in the habitat. After the epochal work of Rowan (1925,1926) on light response of slate coloured Junco, *Junco heymalis,* vast amount of literature has accumulated on photoperiodic regulation of avian breeding cycles and feather renewal process (see reviews: Lofts and Murton, 1968; Farner, 1975; Follett and Robinson, 1980; Wingfield and Farner, 1980; Sharp, 1984a,b; Nicholls *et al*, 1988; Thapliyal and Gupta, 1989; Wingfield and Farner, 1993; Kumar, 1997; Dawson *et al*, 2001; MacDougall-Shackleton and Höhn, 2007; Dawson, 2008, 2015; MacDougall-Shackleton, *et al*, 2009; Ubaka *et al*, 2013; Rani and Kumar, 2014; Nakane and Yoshimura, 2019). Current literature although derived largely from species nesting at mid and high latitudes during spring and/or summer months, describe putative involvement of light and the encephalic photoreceptors in generating circadian and/or circannual responses to help culminate a potential breeding season. Further, owing to the absolute latitudinal accuracy, daylength has been recognised as the 'proximate factor' in entraining circadian/circannual components of the biological clock to click a ‘photosensitive phase’ which appears several hours later to switching on the light.

After the winter solstice, availability of critical daylength (likely to differ between individuals or the mates of a species) triggers endocrine events leading to increase in body mass and gonads which reach maximum and then decrease after few weeks when daylength is still increasing or is more than which has resulted their growth early in the spring season. This response is referred as the onset of photorefractoriness - gonadal and metabolic- which has traditionally been characterized as cessation of stimulatory impact of long/longer daylengths on physiological mechanisms which were responsive to long/longer daylengths earlier in the season. At this stage, further increase in lighting hours may or may not evoke a second or additional response. Now, several lines of evidence suggest that photorefractoriness is not merely a laboratory artefact, but it truly escalates during the post-nuptial phase and is often characterized by down regulation of hypothalamic contents of gonadotrophin releasing hormone-I (GnRH-I), although its expression can be monitored only by experimental means (MacDougall-Shackleton *et al*, 2009; Dawson, 2015; Nakane and Yoshimura, 2019). Interruption of breeding by eventual precipitation of photorefractory states has immense adaptive value in the annual life span of birds since it would ensure feather renewal and repletion of energy stocks depleted during intense breeding activities well before the onset of harsh winter months.

Further, for the sake of clarity, traditionally, the photorefractoriness is designated as ‘absolute’ or ‘relative.’ The former involves cessation of stimulatory impact of long daylengths on neuroendocrine-gonadal and neuroendocrine-metabolic axes, and resumption of light response would necessitate exposure of birds to non-stimulatory daylengths for at least 6-8 weeks (Thapliyal and Lal, 1984a; Devi and Lal, 1994; Nicholls *et al*, 1988; MacDougall-Shackleton *et al*, 2009; Dixit and Singh, 2012; Ubaka *et al*, 2013; Dawson, 2015; Nakane and Yoshimura, 2019). In the latter case, as shown in Japanese quail, *Coturnix coturnix japonica* developed gonads of birds under long/longer daylengths regress following decrease in the prevailing daylengths despite light durations still being more than which had caused their recrudescence earlier in the season (Robinson and Follett, 1982; Follett and Pearce-Kelly, 1990). In such birds, gonads redevelop following transfer to long/longer daylengths (Nicholls *et al*, 1988; Dawson, 2015; Nakane and Yoshimura, 2019). In resident species like Indian weaver bird, *Ploceus* *philippinus* (Thapliyal and Saxena, 1964), wood pigeon, *Columba palumbus* (Lofts *et al*, 1967a), rufous collared sparrow, *Zonotrichia capensis hypoleuca* (Lewis *et al*, 1974) and red-cross bill, *Loxia curvirostra* (MacDougall-Shackleton *et al*, 2006), gonads develop under long daylengths and remain sustained so long daylength is maintained. Such birds lack a photorefractory state and in them, seasonality is maintained by waxing and waning of daylengths (Lofts and Murton, 1968; Thapliyal and Gupta, 1989; Nicholls *et al*, 1988; Dawson, 2008). At low/lower latitudes, though some birds respond to photostimulation, in many, daylengths do not directly influence breeding cycles, instead daylength is used in phasing an autonomous rhythm of reproduction (Thapliyal, 1969, 1978, 1981, 1992; Chandola *et al*, 1983; Sharp, 1984a, 1996; Thapliyal and Gupta, 1989; Dixit and Singh, 2012).

The current scenario states that several bird species from low/lower latitudes are equipped with inherent mechanisms for discriminating small changes in photoperiod and they rely on daylength as the key source of environmental cue to enable them predict most favourable time for raising youngs. However, species occupying different habitats at particular latitude and their ecological congeners inhabiting different latitudes differ at the length of their reproductive periodicity (Perfito *et al*, 2004; Caro *et al*, 2005; Dawson, 2008, 2015). This entails that adaptation plasticity in light response mechanisms of birds is not uniform and may be influenced by non-photoperiodic cues in different habitats in shaping the final reproductive effort (Wingfield, 1983). Red-vented bulbul, *Pycnonotus cafer* is the most widely distributed species (30°N-36°S) which has occupied 37 continental locations outside its native ranges, but its breeding biology is relatively less explored. In certain locations, reproductive periodicities have been described to differ between years owing presumably to the habitat quality (McCann, 1931; Dutt, 1932; Lamba, 1968; Vijayan, 1980; Bhatt and Kumar, 2001; Walker, 2008; Acharya and Vijayan, 2009; Balkrishnan, 2010; Prajapati *et al*, 2011; Rao *et al*, 2013; Awais *et al*, 2014; Radhakrishnan and Ashokan, 2014, 2015; Yadav *et al*, 2018; Thiabault *et al*, 2018a,b, 2019; Zohaib *et al*, 2021). Further, limited findings have portrayed seasonal variations in daylengths as the key regulator of breeding and moulting cycles (Lal and Thapliyal, 1982a). However, a systematic approach on photoperiodic response of red-vented bulbul is awaited. In the present study, we have examined seasonal sensitivity of neuroendocrine-gonadal and neuroendocrine-metabolic axes to photostimulation in red-vented bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami, (26°N), Zunheboto district, Nagaland State.

MATERIALS AND METHODS

Birds used in the present study were caught locally and acclimatized to laboratory conditions for a minimum duration of fortnight before their use on the present set of experiments. Altogether 03 experiments were made.

Experiment-I: This experiment was made to assess light response of birds under simulated long, medium and short daylengths. During the first week of December 2018, 60 birds were procured from local bird catchers. Birds were sexed through exploratory laparotomy and 05 groups of male and 05 groups of females were established with each group consisting of 06 birds only. Four groups of male and 04 groups of females were separately transferred to simulated 20L/4D, 16L/8D, 12L/12D and 8L/16D daylengths. The remaining groups of male and female birds were maintained under natural lighting conditions and served as the control group. Body mass, gonad size and feather moult of individual birds in all the 05 groups were maintained on monthly basis until September 2018.

Experiment- II: This experiment was performed to assess seasonal sensitivity of the photoperiodic response system to simulated long daylengths (16L/8D and 20L/4D) during different reproductive and metabolic states. Since under constant 8L/16D daylength gonads of both the sexes of bulbuls did not develop and at 12L/12D daylength only partial gonadal development had occurred and that trends in light responses of male and female birds were essentially similar, further experiments were taken up only on male birds. Every month, starting from January-November 2019, groups of 05 male birds were separately exposed to constant 16L/8D and 20L/4D photoperiods. A group of 05 birds was maintained under natural lighting conditions to serve as the control. Monthly records on testicular size *in situ* and body mass of individual birds were kept regularly.

Experiment- III: This part of the experiment had prime focus on the query whether gonads and body mass of bulbuls on long days decline owing to the development of photorefractory states? If so, then what is the nature of photorefractoriness: absolute or relative? Five male birds after they became refractory to constant 16L/8D photoperiod were transferred to constant 24L/0D daylength. The control group of 05 birds was retained on 16L/8D daylength. Records on body mass and left testis size *in situ* of both the groups were kept at monthly intervals for two consecutive months.

Light treatments were provided in light-proof boxes fitted with fluorescent tubes to deliver light intensity at ~400lux at the perch level. Light schedules were controlled through automatic timers (DIEHL MIII-S; 16L/8D: light on at 6 hr; off at 22 hr; 8L/16D: light on at 6 hr; off at 14 hr). Food, husbandry conditions, techniques followed for recording body mass, gonads and feather moults were as detailed in Chapter-I. At termination of an experiment, birds were rehabilitated to natural conditions and were then set free.

Data were analysed using one way independent measure of ANOVA. Between group comparisons were made using Student's *'t'* test.

RESULTS

Experiment-I: Effect of Constant Long and Short Photoperiods on Body Mass, Gonads and Feather Moult:

Gonads: In captive birds maintained under NDL, testicular recrudescence began during January-February, reached at peak (108.16±10.70 mm^3) in June and thereafter testes declined to reach a low value in September. One way independent measure of ANOVA revealed a significant ($F_{(9,50)}$=21.41, $p<0.0001$) within group variation in testicular size of these birds. Mean testicular size of birds during June was significantly higher compared with within group values of other months (Table 10 Fig.8 A). Transfer of birds to constant 16L/8D or 20L/4D daylengths during December led to significant within group changes (16L/8D: $F_{(9,50)}$= 35.67, $p<0.0001$; 20L/4D: $F_{(9,50)}$=59.66, $p<0.0001$) in testicular sizes of birds. In both groups of birds, testes grew significantly ($p<0.001$) within a month, attained peak in 3rd month (16L/8D: 63.05±7.05 mm^3; 20L/4D: 69.94±7.88 mm^3) and thereafter declined slowly to reach a low value within next 3-4 months. Mean testicular size in 16L/8D or 20L/4D birds in 3rd month was significantly higher as compared to within group values of other months (Table 10, Fig. 8A). Under constant 12L/12D light schedule within group ANOVA revealed a significant change in testicular size ($F_{(9,50)}$= 4.52, $p<0.001$). Testes of birds had partial but significant increase within first two months of exposure and thereafter testes remained partially developed until September when the experiment was terminated. Testicular sizes on day 0 and 01 month after photostimulation by

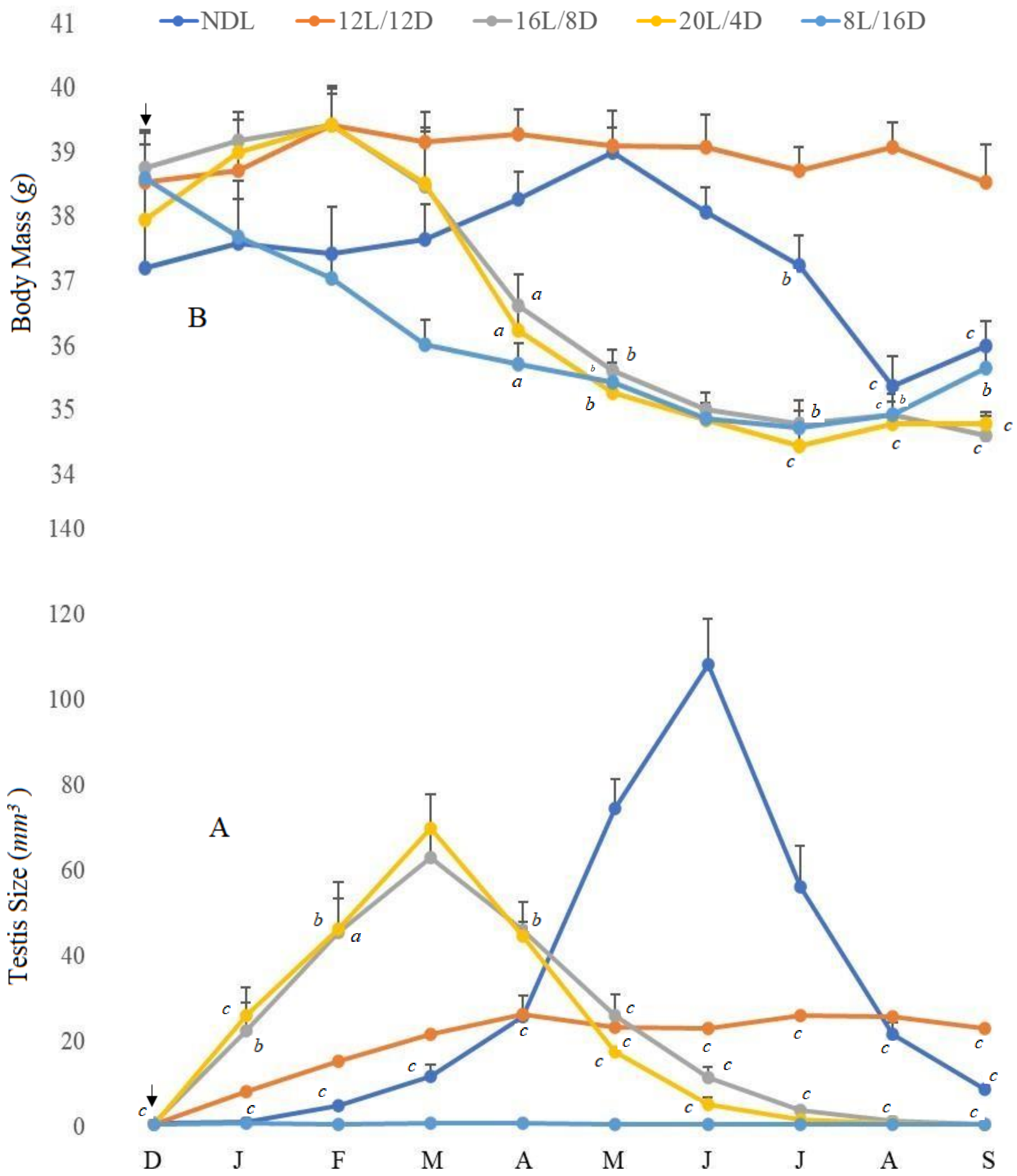

Fig8: Effect of simulated Long and Short Daylengths on Testicular and Body Mass Cycles of Red-vented Bulbul, *Pycnonotus cafer stanfordi*. Testis Size NDL: F($_{9,50}$)=21.41, p<0.0001; 12L12D: F($_{9,50}$)= 4.52, p<0.001; 16L/8D: F($_{9,50}$)=5.67, p<0.0001; 20L/4D: F($_{9,50}$)=59.66,P<0.0001; 8L/16D: F($_{9,50}$)= 0.42, (ANOVA).*a,b,c* differ from the highest value within group at p<0.05; <0.01 & <0.001 level respectively;Body Mass NDL: F($_{9,50}$)=10.81, p<0.0001; 12L12D: F($_{9,50}$)= 0.47; 16L/8D: F($_{9,50}$)=7.30, p<0.0001; 20L/4D: F($_{9,50}$)=11.00, P<0.0001; 8L/16D: F($_{9,50}$)= 6.42, p<0.0001 (ANOVA). *a,b,c* differ from the highest value within the group at p<0.05; <0.01 & <0.001 level respectively (Student's *'t'* test).

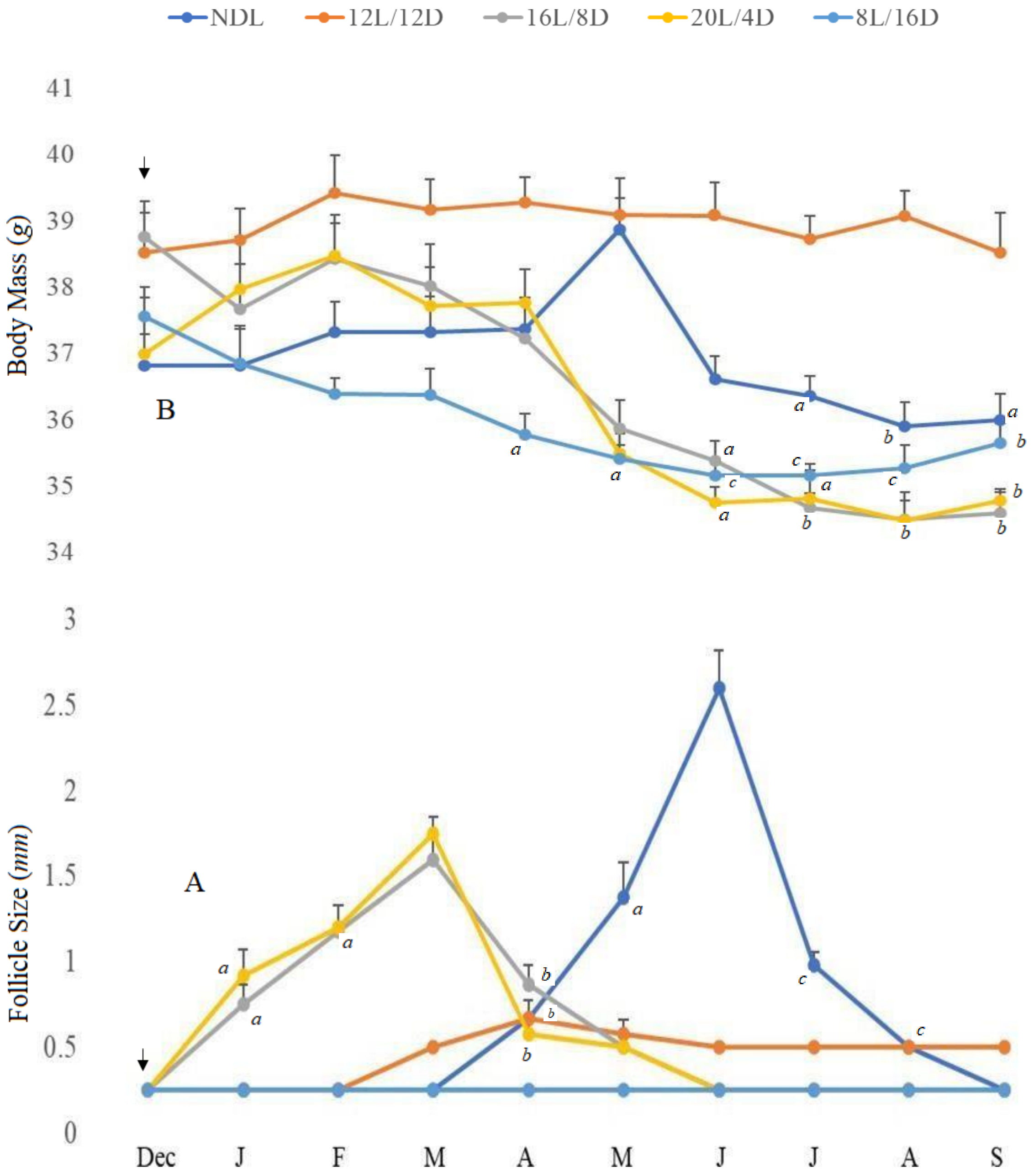

Fig.9: Effect of Simulated Long and Short Daylengths on Follicular Cycle of Red-vented bulbul, *Pycnonotus cafer Stanfordi*. Follicular Size, NDL: F($_{9,50}$) 24.93, $p<0.0001$; 12L12D: F($_{9,50}$)= 5.33, $p<0.0001$; 16L/8D: F($_{9,50}$)=23.96, $p<0.0001$; 20L/4D: F($_{9,50}$)=24.15, $P<0.0001$; 8L/16D: F($_{9,50}$)= 0.14, (ANOVA). *a,b,c* differ from the highest value within the group at $p<0.05$; <0.01 & <0.001 level respectively (Student's *'t'* test).;Body Mass, NDL: F($_{9,50}$)=3.57, $p<0.001$; 12L12D: F($_{9,50}$)= 0.36,; 16L/8D: F($_{9,50}$)=12.14, $p<0.0001$; 20L/4D: F($_{9,50}$)=10.68 $P<0.0001$; 8L/16D: F($_{9,50}$)= 6.42, $p<0.0001$ (ANOVA). *a,b,c* differ from the highest value within the group at $p<0.05$; <0.01 & <0.001 level respectively (Student's *'t'* test).

12L/12D were significantly less compared with values of other months. Within group comparisons of testes of birds exposed to fixed duration of 8L/16D showed no significant variation during the treatment period ($F_{(9,50)}$= 0.42, non-significant) (Table 10, Fig. 8A). In NDL birds, ovarian follicles remained small until February, developed slowly between March-May, attained peak in June (2.60±0.22 mm) and thereafter declined to regress by September (Table 12, Fig. 9A). One way within group comparison of data revealed a significant change in follicular size of birds ($F_{(9,50)}$= 24.93, $p<0.0001$). Barring the follicular size in May, average follicular diameter in the month of June measured significantly more compared with values of other months. Ovarian follicles developed rapidly in birds transferred to constant 16L/8D ($F_{(9,50)}$= 23.96, $p<0.0001$) and 20L/4D ($F_{(9,50)}$= 24.15, $p<0.0001$), follicles attained peak in 3rd month (16L/8D: 1.60±0.15mm; 20L/4D: 1.75±0.10 mm) and thereafter declined slowly to reach a non-measurable size in the next two months. Peak follicular size of birds under constant 16L/8D and 20L/4D measured significantly more compared to within group values in other months (Table 12, Fig. 9A). A significant increase in follicular size over its initial value ensued in birds exposed to constant 12L/12D photoperiod. However, follicles of these birds remained only partially developed until September when the experiment was terminated. Mean follicular sizes of this group on day 0, 01 and 02 months after light treatments, were significantly less compared with values of other months (Table 12, Fig. 9A). Seasonal follicular development was inhibited in birds transferred to constant 8L/16D photoperiod during December (Table 12, Fig. 9A). Maximum testicular size of NDL birds in the month of June (108.16±10.70 mm^3) was significantly ($p<0.05$) more as compared to testicular peak of birds under constant 16L/8D (63.05±7.05 mm^3 & 20L/4D (69.94±7.88 mm^3) photoperiods. Similarly, maximum follicular size of NDL group in June (2.60±0.22 mm) measured significantly more than follicular sizes of birds under 16L/8D (1.60±0.15 mm) and 20L/4D (1.75±0.10 mm) daylengths (Table 12, Fig. 9A).

Body Mass: One way ANOVA revealed a significant within group change ($F_{(9,50)}$=4.81, $p<0.01$) in body mass of birds maintained under NDL. Body mass was maximum in May (39.01±0.37g) and minimum (35.38±0.46g) in August. From June to September period, mean body mass of birds was significantly less as compared to peak value of May. Mean Body mass of 16L/8D ($F_{(9,50)}$=3.30, $p<0.05$) and 20L/4D ($F_{(9,50)}$=3.1 $p<0.05$) groups experienced significant changes during the treatment period. Under both these photo-schedules, birds weighed maximum after 02 months of exposure followed by gradual decline. Further, maximum body mass of these groups

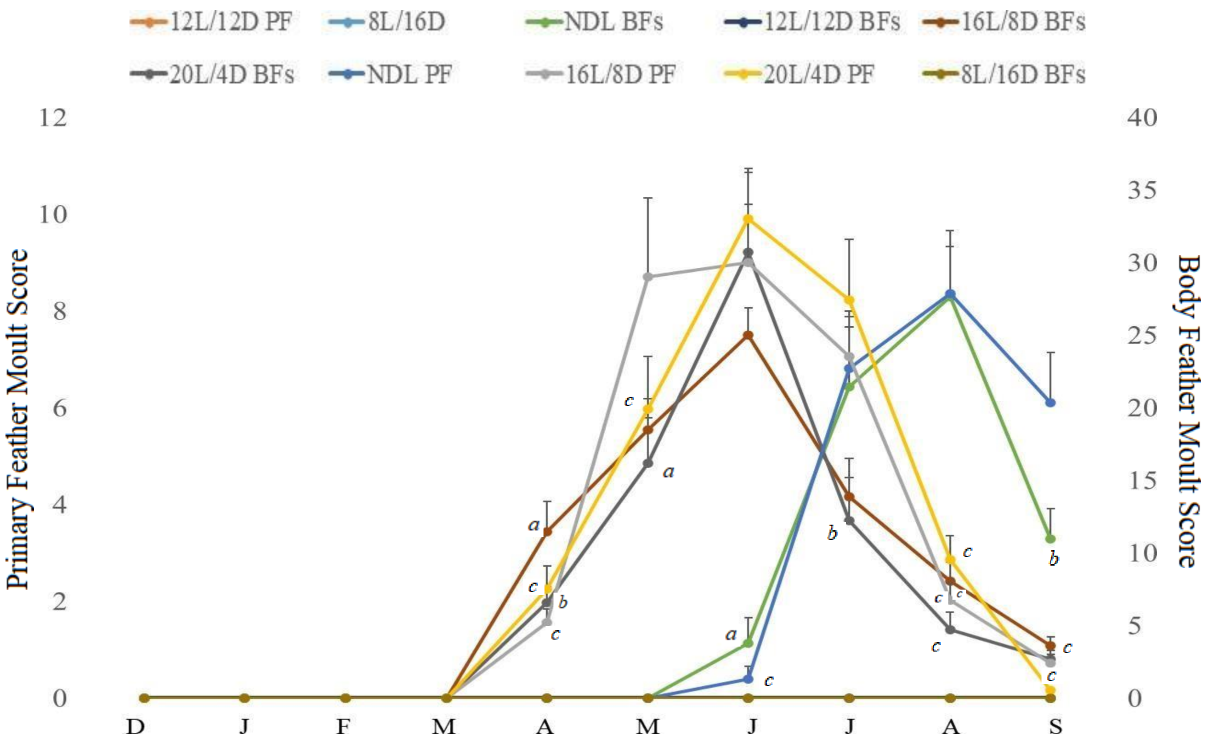

Fig.10: Effect of simulated Long and Short Daylengths on Feather Moult of Male Red-Vented Bulbul, *Pycnonotus cafer Stanfordi*. Values are Mean±*SE*, n= 6 in each group.*a,b,c* differ from the highest value within the group at p<0.05; <0.01 & <0.001 level respectively (Student's *'t'* test).

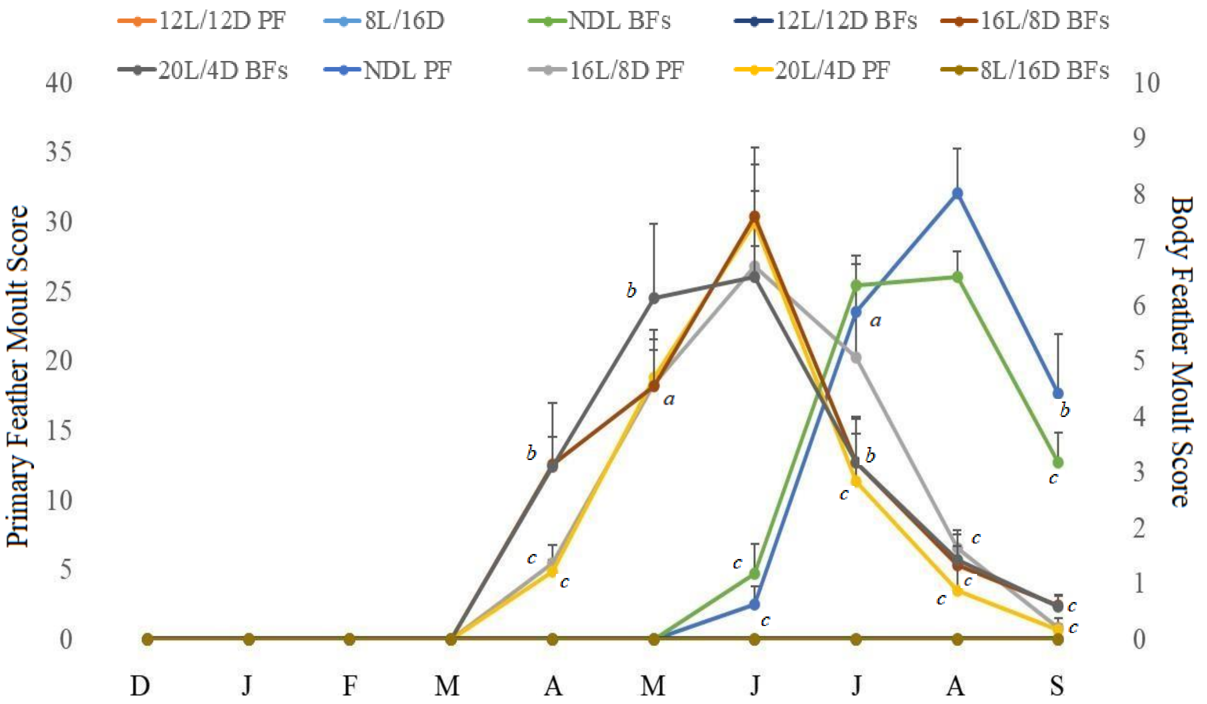

Fig11: Effect of Simulated Long and Short Daylengths on Feather Moult of Female Red-vented Bulbul, *Pycnonotus cafer stanfordi*. Values are Mean±*SE*, n= 6 in each group. *a,b,c* differ from the highest value within the group at p<0.05; <0.01 & <0.001 level respectively (Student's *'t'* test).

after 02 months were significantly more as compared to values from 04-09 months after exposure (Table 11, Fig. 8 B). No significant change was registered in body mass of birds exposed to constant 12L/12D daylength ($F_{(9,50)}$=0.47) (Table 11, Fig. 8 B). Body mass of birds under fixed duration of 8L/16D underwent significant within group change ($F_{(9,50)}$=3.42, $p<0.05$) during the treatment period. After 03-09 months of chronic exposures to short daylength, mean body mass of this group was significantly less compared to initial value during December (Table 11, Fig. 8 B).

One way ANOVA in NDL females showed a significant within group changes in body mass of birds ($F_{(9,50)}$=3.57, $p<0.01$). Body mass of females was maximum in May (38.88±0.47g) and minimum (35.90±0.37g) in August. Mean body mass during May was significantly more compared with values of July-September months (Table 13, Fig. 9 B). One way ANOVA revealed significant within group alterations in body mass of birds exposed to fixed durations of 16L/8D ($F_{(9,50)}$=3.14, $p<0.05$) and 20L/4D ($F_{(9,50)}$=2.68, $p<0.05$) photoperiods. Mean body mass of 16L/8D group was maximum (38.43±0.54g) after 02 months and minimum (34.50±0.29) after 07 months. Maximum value after 02 months of 16L/8D treatment was significantly higher as compared to values from 05-09 months after exposure (Table 13, Fig. 9 B). Maximum body mass of birds after 02 months under 20L/4D differed significantly from values of 06-09 months after light treatment (Table 13, Fig. 9 B). No significant within group variation was witnessed in birds exposed to constant 12L/12D ($F_{(9,50)}$=0.36). However, significant within group change occurred in body mass of birds kept under constant 8L/16D photoperiod ($F_{(9,50)}$=3.42, $p<0.01$). Mean body mass of birds from 04-09 months under 8L/16D differed significantly as compared to December value (Table 13, Fig. 9 B).

***Feather Moult* :** In NDL males, primary feather (PFs) and body feather (BFs) moults started in June, were maximum in August (moult scores: PFs, 27.86±3.25 & BFs, 8.30±1.36) and thereafter declined slowly. Mean PFs moult score in June was significantly less as compared to values from July-September (Table 14, Fig. 10). BFs moult scores in June and September were significantly less compared to maximum score in August (Table 14, Fig. 10). PFs moult in birds exposed to constant 16L/8D or 20L/4D began in 04th month of exposure, attained peak (16L/8D: 30.00±4.00; 20L/4D: 33.01±3.50) in 06th month and thereafter declined slowly (Table 14, Fig. 10). BFs moult under constant 16l/8D or 20L/4D began in 04th months of exposure, was maximum (16L/8D: 7.50±0.56; 20L/4D: 9.21±1.65) in 06th month and thereafter declined slowly. No feather moult could be seen in birds maintained on constant 12L/12D or 8L/16D daylengths (Table 14, Fig. 10).

In NDL females PFs and BFs started in June, were highest in August (moult scores: PFs, 32.03±3.22 & BFs, 6.50±0.46) and declined in September (Table 15, Fig.11). In birds maintained on constant 16L/8D or 20L/4D, PFs and BFs began in 04th month of light treatment, attained peak (moult scores: PFs, 16L/8D: 26.80±5.36; 20L/4D: 29.88±4.20 & BFs:16L/8D: 7.60±1.23; 20L/4D: 6.50±0.56 in 06th month and thereafter declined slowly (Table 15, Fig.11). No feather moult ensued in groups maintained on constant 12L/12D or 8L/16D daylengths (Table 15, Fig.11).

Experiment- II: ***Response of Light responding system to photostimulation during different reproductive and metabolic states***:

Testicular size: Testes of NDL birds continued developing through Feb-May, reached at peak in June (105.18±11.19 mm^3) and thereafter declined reaching low value in August at the time of termination of the experiment. Transfer of birds from NDL to constant 16L/8D or 20L/4D daylengths in January resulted in rapid testicular growth within a month. Testes reached at maximum (16L/8D: 76.82±8.22 mm^3; 20L/4D: 80.56±9.83 mm^3) in 3rd month of exposure which was followed by regression (Table 16, Fig.12). The rate of testicular growth and the maximum sizes attained under 16L/8D and 20L/4D daylengths were significantly not different from each other. Exposure of birds to 16L/8D and 20L/4D daylengths in February led to significant increase in testicular size within a month; testes grew maximally (16L/8D: 86.42±8.21 mm^3; 20L/4D: 102.56±12.23 mm^3) in the 2nd month and thereafter regressed to minimum by 6th month. Trends in testicular growth and the maximum size achieved in testes of birds under both the photo-schedules were significantly not different (Table 17, Fig.13).

Similar response to photostimulation was witnessed in testes of birds shifted from NDL to constant 16L/8D or 20L/4D daylengths in March. Testicular peak appeared 2 months after photostimulation (16L/8D: 98.58±10.15 mm^3; 20L/4D:110.64±9.26 mm^3) which was followed by regression in 3rd month onwards (Table 18, Fig.14). Following light treatment in April, testicular development occurred in close parallelism with NDL birds with testicular peak (16L/8D:115.86±9.86 mm^3; 20L/4D: 119.52±11.83 mm^3) appearing at the end of 2nd month coinciding testicular peak with birds under natural lighting followed by rapid decline (Table 19, Fig.15). Similarly, exposure of birds to constant 16L/8D and 20L/4D during May had no effects on the development of testes as compared to NDL birds but testicular regression was rapid compared with NDL birds (Table 20, Fig.16). Constant 16L/8D or 20L/4D had no effects on the maximally developed testes of birds in June as gonads regressed together in photostimulated groups and NDL birds (Table 21, Fig.17). Further, exposure of NDL birds to constant 16L/8D and 20L/4D daylengths in July and August failed in preventing testicular regression (Table 22-23, Fig.18, 19). Photostimulation by 16L/8D and 20L/4D daylengths in the month of September had no significant effects on regressed testes of birds (Table 24, Fig. 20). In October, testes of birds transferred to constant 16L/8D or 20L/4D daylengths grew significantly in 03 birds (60%) and no response in the remaining 02 (40%) birds in the group. Mean testicular volumes of all the birds in the group were at 2.60±0.67mm^3 (16L/8D) and 3.61±1.13mm^3 (20L/4D) respectively which were followed by instant regression. Mean testicular size of birds after one month in both the photoschedules were significantly more as compared to NDL birds in the corresponding month (Table 25, Fig.21). Significant testicular growth ensued in 80% as 04 out 05 birds responded to photostimulation by constant 16L/8D or 20L/4D daylengths in November. Mean testicular size of long day birds after 01 (16L/8D: 16.34±2.62 mm^3; 20L/4D: 17.20±5.91mm^3) and 02 months (16L/8D: 10.72±3.96mm^3; 20L/4D: 14.34±3.40 mm^3) were significantly more as compared to NDL birds in the corresponding months. Testes of birds in both the groups started regressing after one month and reached to small size in 3rd month of exposure to long days. Mean testicular size of birds after 01 and 02 months of exposures to long daylengths were significantly more compared to values of NDL birds in corresponding months (Table 26, Fig. 22).

Body Mass: Body mass of NDL birds measured maximum in May (38.56±0.81g) and minimum in August (35.88±0.29g). Mean body mass of birds in the month of May measured significantly more as compared to the value in July and August months. Transfer of birds from NDL to constant 16L/8D or 20L/4D during January and February had no significant influence on body mass of birds in the first two months. However, during 2nd and 3rd month onwards body mass of these groups began declining and in 6th and 7th months mean body mass were significantly less as compared to values after one month of light treatment (Table 16,17, Figs. 12-13). Exposure of birds to constant 16L/8D and 20L/4D in March and April had no significant effects on body mass as compared to NDL group (Tables 18, 19, Figs.14-15). Similarly, no significant difference

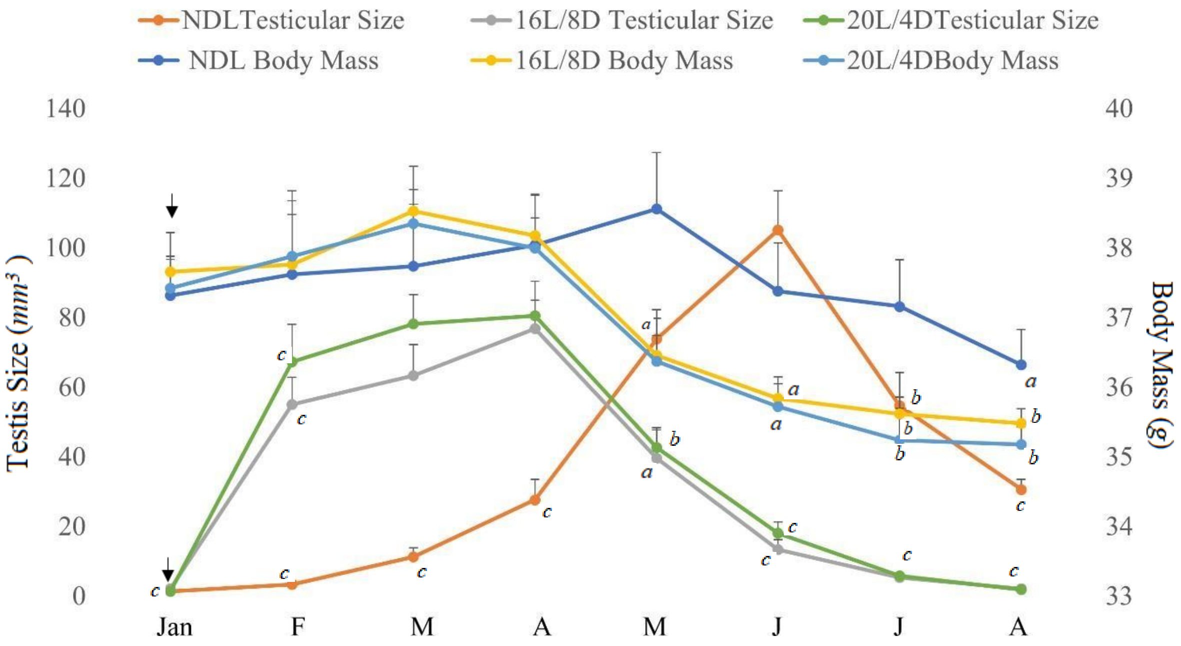

Fig12: Effect of Constant 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented bulbul *Pycnonotus cafer stanfordi*. Values are Mean±*SE*, n= 5 in each group. *a*,*b*,*c* differ from the highest value of indices within the group at p<0.05; <0.01 & <0.001 level respectively (Student's *'t'* test).

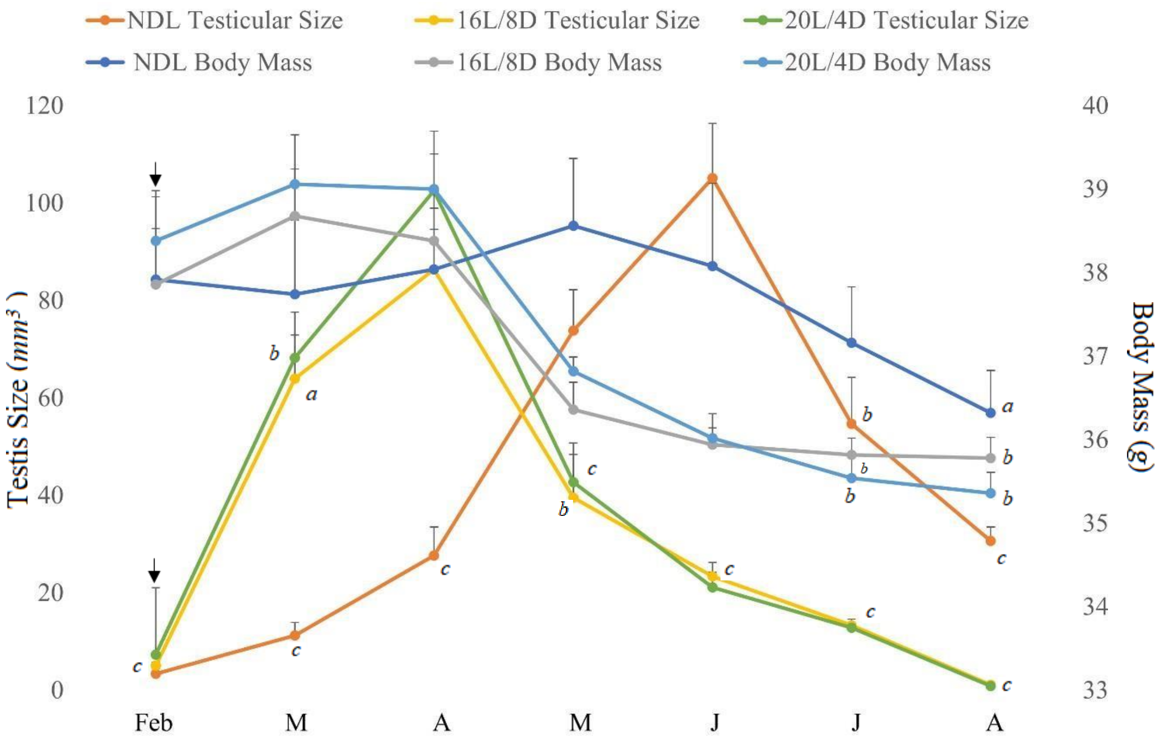

Fig.13: Effect of Constant 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented bulbul *Pycnonotus cafer stanfordi*. Values are Mean±*SE*, n= 5 in each group. *a*,*b*,*c* differ from the highest value within the group at p<0.05; <0.01 & <0.001 level respectively (Student's *'t'* test)

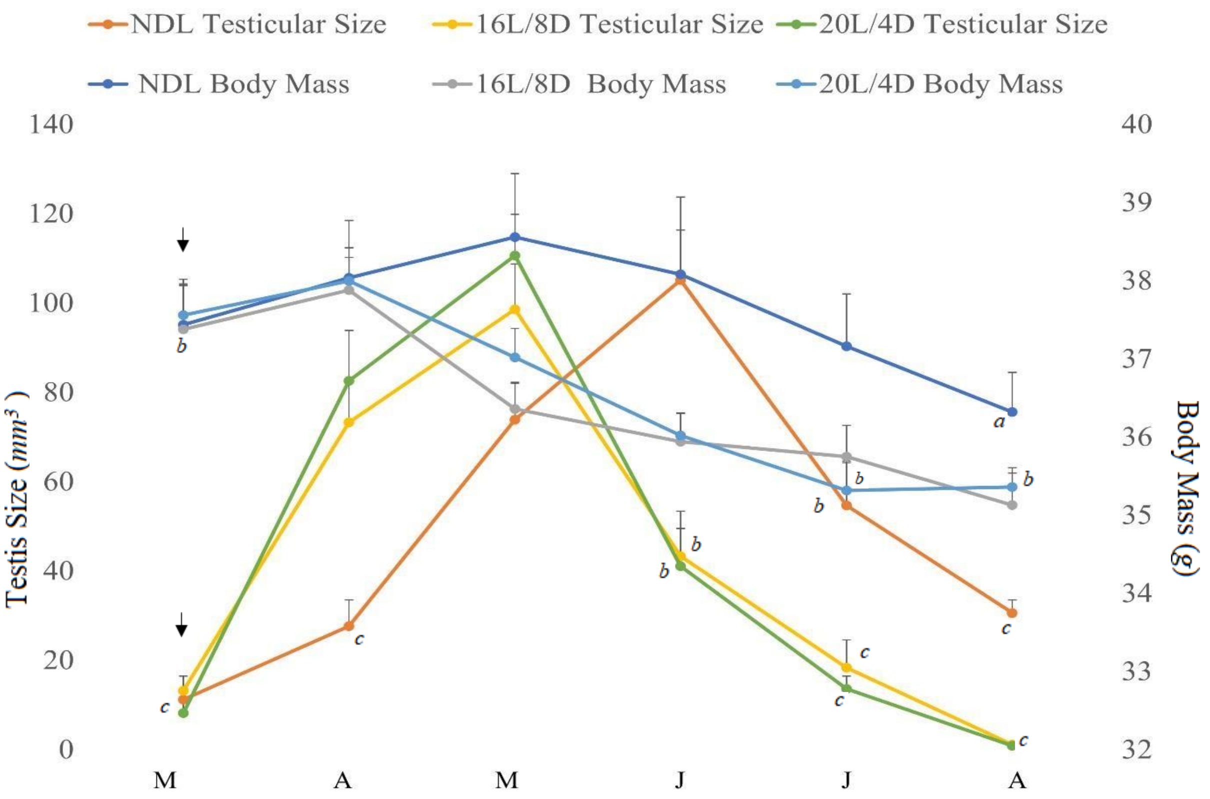

Fig14: Effect of Constant 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented bulbul *Pycnonotus cafer stanfordi*, Values are Mean±*SE*, n= 5 in each group.*a,b,c* differ from the highest value within the group at p<0.05; <0.01 & <0.001 level respectively (Student's *'t'* test).

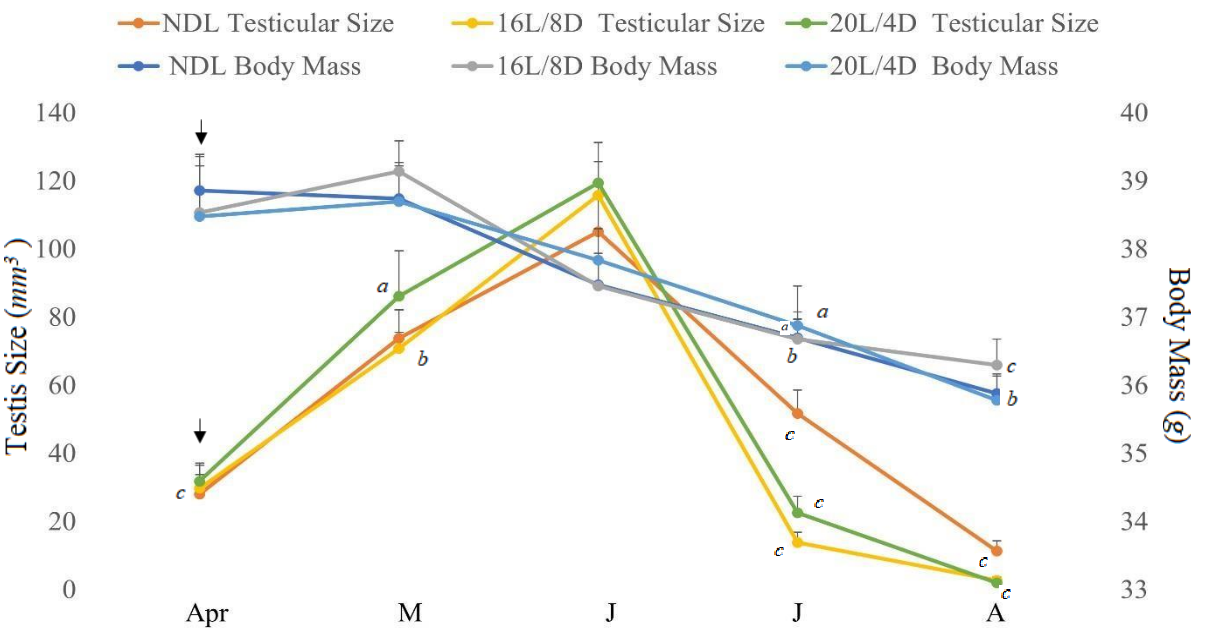

Fig15: Effect of Constant 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented bulbul, *Pycnonotus cafer stanfordi*. Values are Mean±*SE*, n= 5 in each group. *a,b,c* differ from the highest value within the group at p<0.05; <0.01 and <0.001 level respectively (Student's *'t'* test).

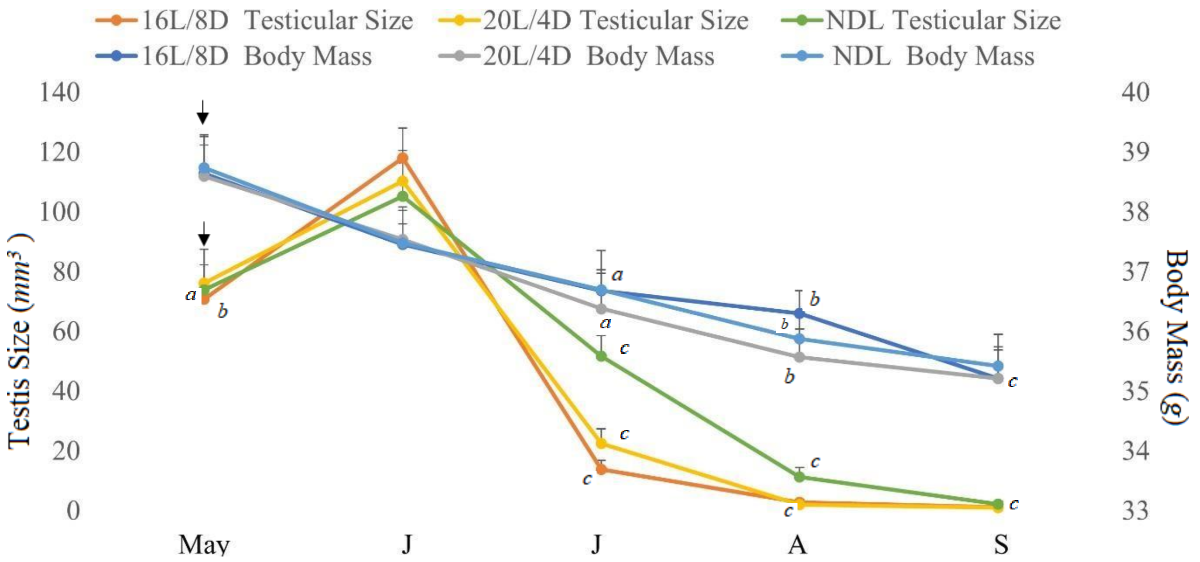

Fig16: Effect of Constant 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented bulbul, *Pycnonotus cafer stanfordi.* Values are Mean±*SE*, n= 5 in each group. *a,b,c* differ from the highest value within the group at $p<0.05$; <0.01 & <0.001 level respectively (Student's *'t'* test).

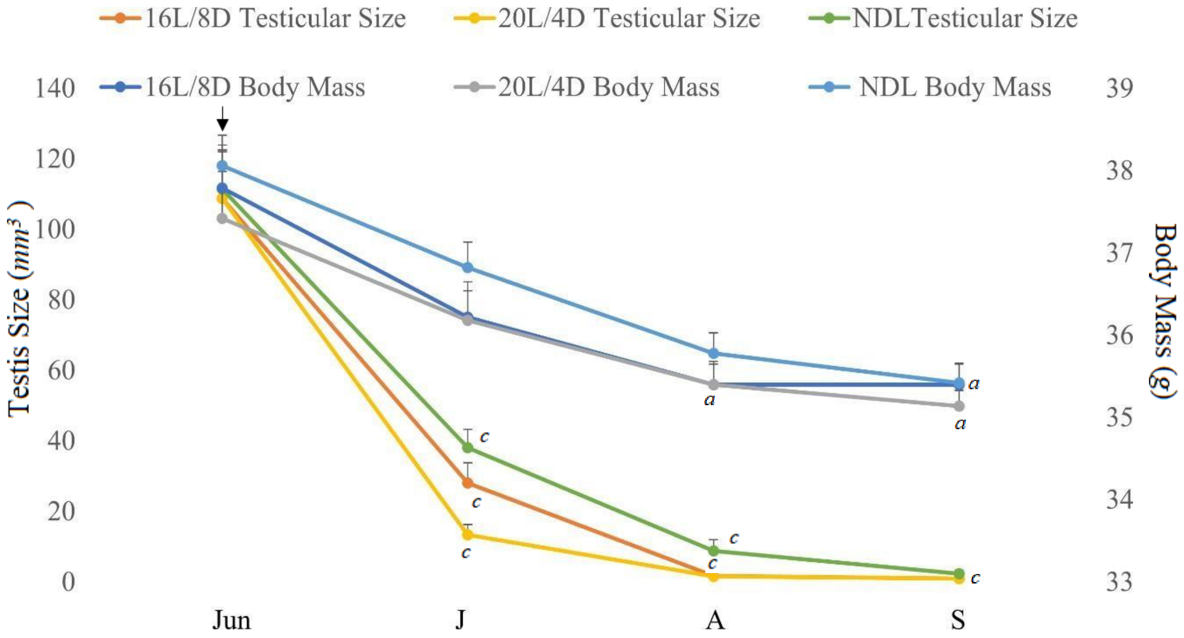

Fig17: Effect of constant 16L/8D photoperiod on Testicular size and Body Mass of Red-vented bulbul, *Pycnonotus cafer stanfordi.* Values are Mean±*SE*; n=5 in each group. *a,b,c* differ from the value of June at $p<0.05$;<0.01 and <0.001 level respectively (Student's *'t'* test).

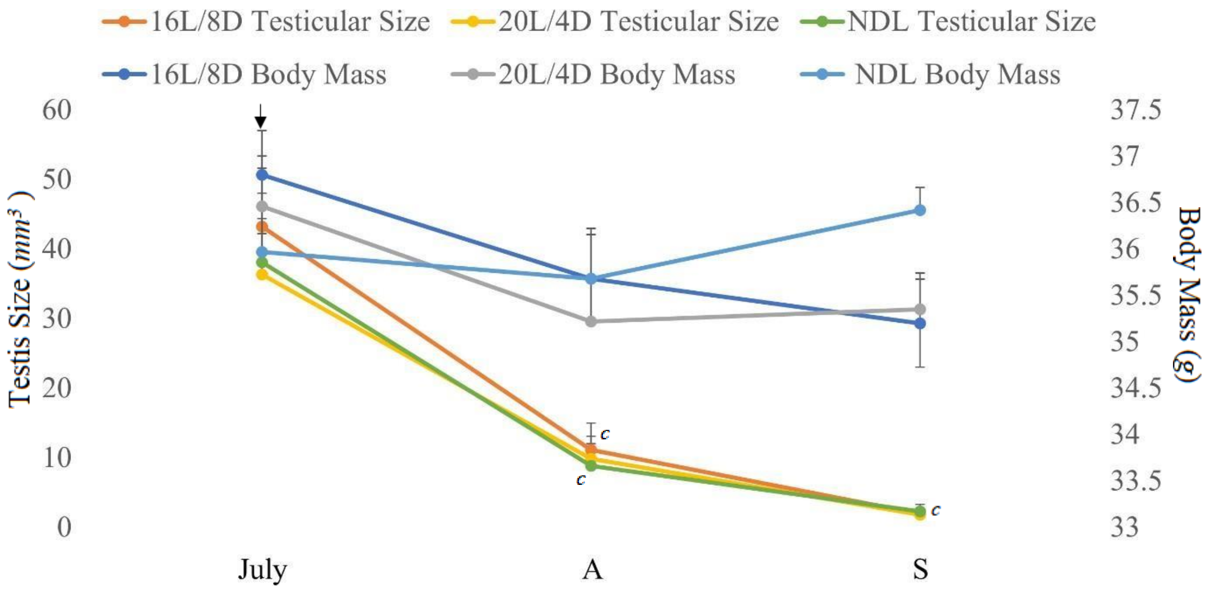

Fig18: Effect of constant 16L/8D photoperiod on Testicular size and Body Mass of Red-vented bulbul, *Pycnonotus cafer stanfordi*. Values are Mean±*SE*; n=5 in each group. *c* differs from the value of July within the group at $p<0.001$ level (Student's *'t'* test).

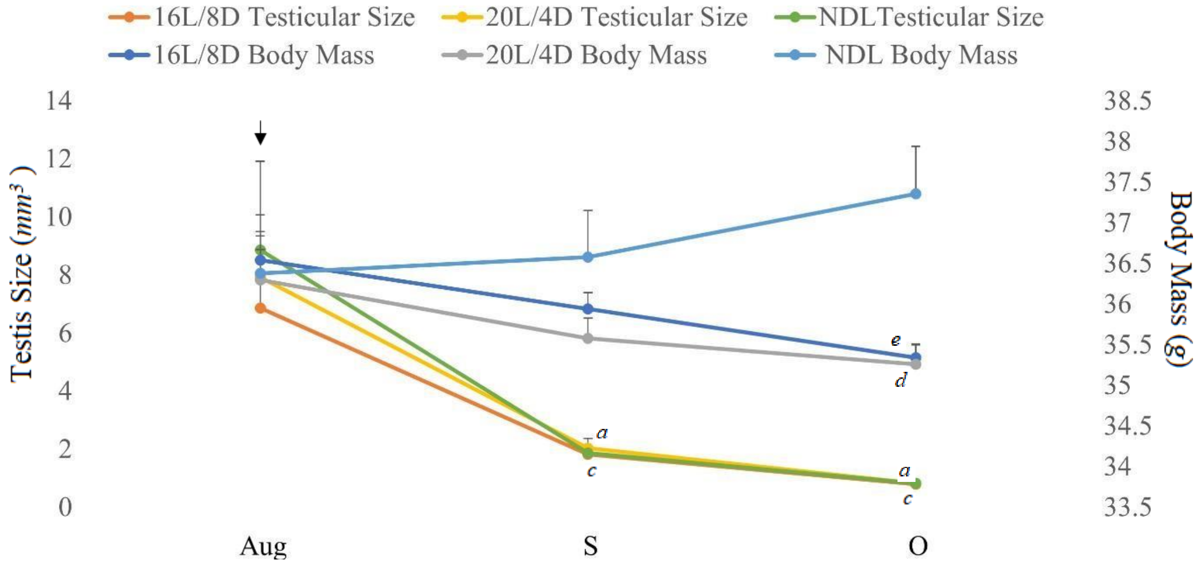

Fig19: Effect of Simulated Long Daylengths on Testicular size and Body Mass of Red-vented bulbul, *Pycnonotus cafer stanfordi*.Values are Mean±*SE*; n=5 in each group. *a*,*c* differ from within group testicular sizein August at $p<0.05$ and $p<0.001$ level respectively. *d*,*e* differ from within group body mass in August at $p<0.05$ and $p<0.001$ respectively (Student's *'t'* test).

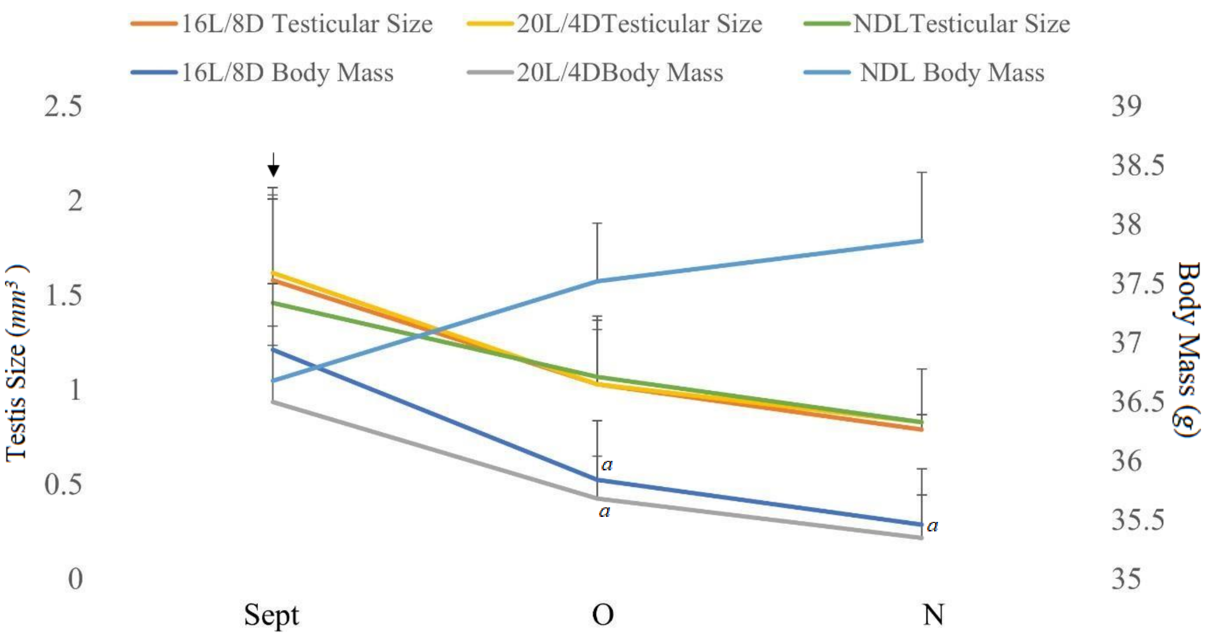

Fig20: Effect of Simulated Long Daylengths on Testicular size and Body Mass of Red-vented bulbul, *Pycnonotus cafer stanfordi*. Values are Mean±*SE*; n=5 in each group. *a* differs from the body mass of NDL group in corresponding months at $p<0.05$ (Student's *'t'* test).

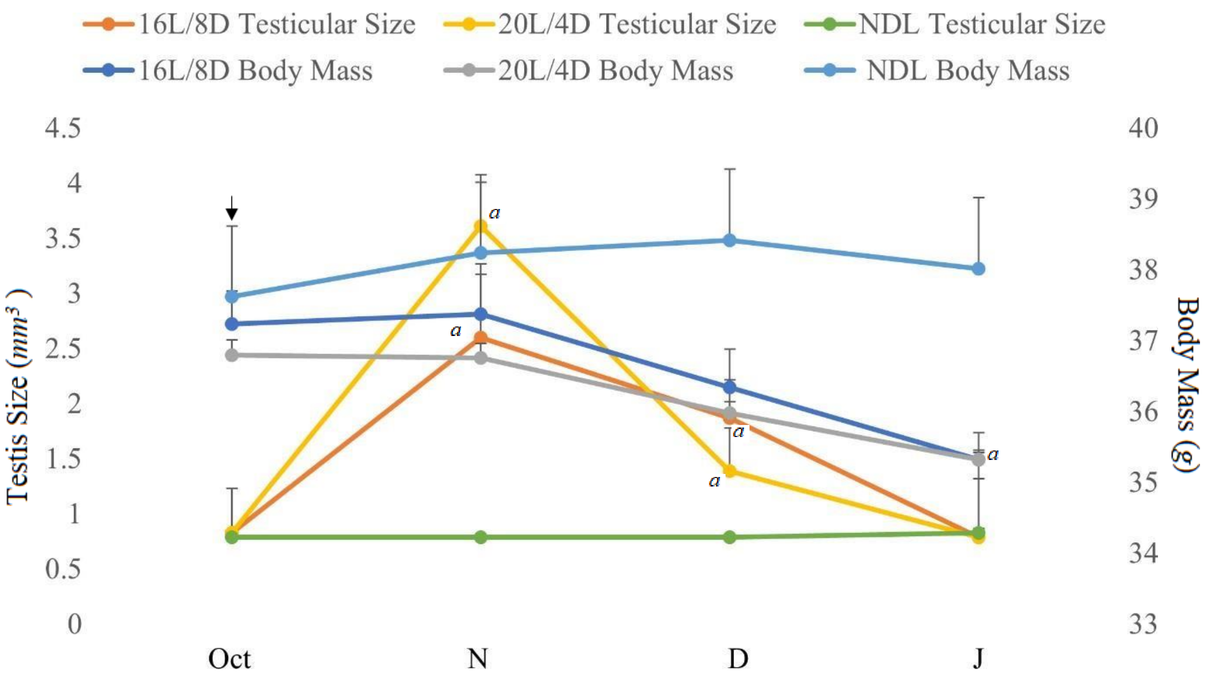

Fig.21: Effect of Simulated 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented bulbul, *Pycnonotus cafer stanfordi*. Values are Mean±*SE*; n=5 in each group. *a* differs from the value of NDL birds in corresponding months at $p<0.05$ level (Student's *'t'* test).

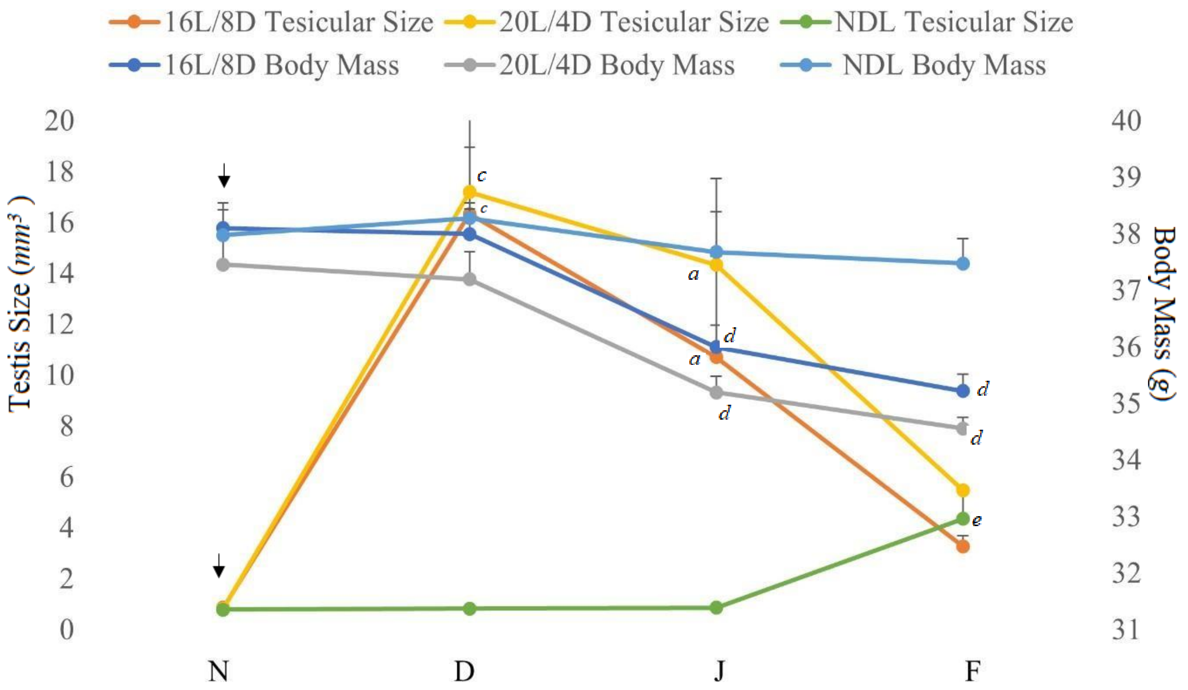

Fig.22: Effect of Simulated Long days on Testicular size and Body Mass of Red-vented bulbul, *Pycnonotus cafer stanfordi*. Values are Mean±*SE*; n=5 in each group *a*,*c* differ from the value of NDL birds in corresponding months at $p<0.05$ and $p<0.001$ level respectively. *e* differs from within group value of testes of NDL birds $p<0.05$. *d* differs from the mean body mass of November birds within the group at $p<0.05$ level (Student's *'t'* test).

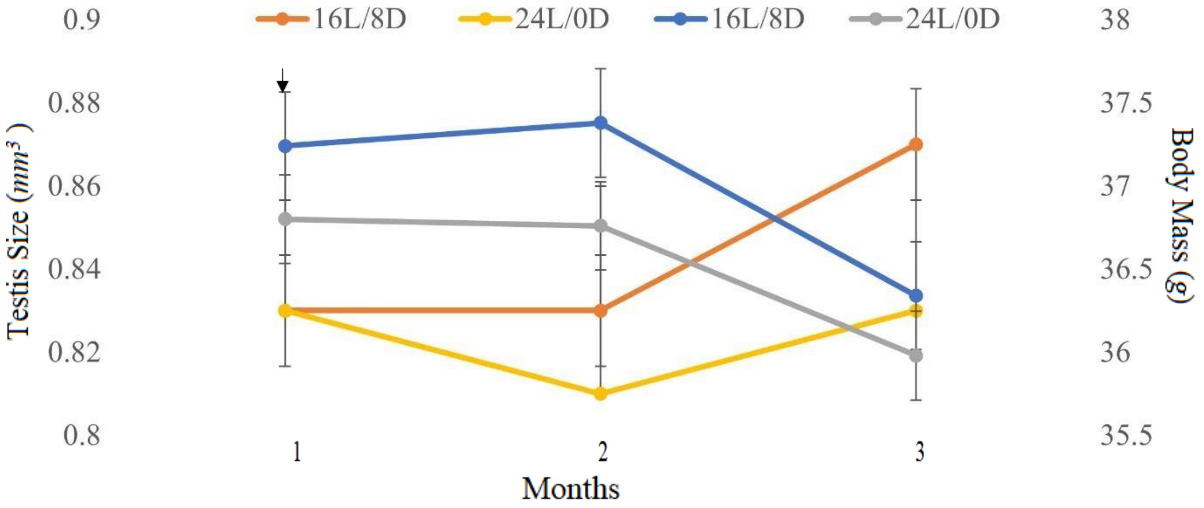

Fig.23: Effect of 24L/0D on Testicular size and Body Mass of Photorefractory, Red-vented bulbul, *Pycnonotus cafer stanfordi*. Values are Mean±SE; n=4 in each group.

was observed in body mass of birds transferred from NDL to constant 16L/8D or 20L/4D from May- September when compared with NDL group (Table 20-24, Figs.16-20). However, in October, mean body mass of NDL birds was more as compared to light treated groups (Table 25, Fig. 21). No significant change occurred in body mass of birds exposed to constant 16L/8D or 20L/4D daylengths during November as compared to NDL group (Table 26, Fig. 22).

Experiment- III: Influence of continuous illumination on testis and body mass of refractory birds under constant 16L/8D*:* Transfer of birds to constant 24L/0D after they became refractory to 16L/8D had no significant influence on regressed gonads and/ or body mass of birds (Table 27, Fig. 23).

DISCUSSION

Results of present study show that body mass and gonad development cycles of captive red-vented bulbuls, *Pycnonotus cafer stanfodi* followed solar cycle with maxima coinciding longest daylength (13hr, 30min) in the month of June. Initiation of gonadal recrudescence in birds around January/February (daylength ~11hrs) denotes that critical daylength necessary for booting neuroendocrine-gonadal axis of red-vented bulbul rests at/around 11hrs. However, rapid development of gonads and maturation of reproductive system would necessitate heightened functional tone of hypothalamic drive to manifest augmented release of GnRH-I and/or pituitary gonadotrophins from the hypothalamo-hypophyseal complex which occur normally under the influence of naturally increasing daylengths from 12-13.5 hr between Spring/vernal equinox and summer solstice. Rapid development of gonads under 16L/8D and 20L/4D, partial gonadal enlargement under 12L/12D and the lack of gonadal recrudescence in birds kept on fixed duration of 8L/16D strongly advocate in favour of such a supposition. Rapid development of ovarian follicles in NDL birds during April-June period further strengthens this view. These features denote that in red-vented bulbuls increase in natural lighting hours from 12-13.5 hr serve as a zeitgeber to synchronise seasonal responses in different physiological and behavioural attributes. Response of birds to constant long (16L/8D or 20L/4D) and short (8L/16D) photo-schedules during December (quiescent phase) is in conformity with the observation on light response of red-vented bulbuls at Varanasi (25^{o}N) wherein testes of birds transferred from NDL to fixed duration of 15L/9D in December developed rapidly to peak in 3rd month followed by slow regression but gonads of birds failed to develop under constant 9L/15D daylength (Lal and

Thapliyal (1982a). These customary responses of red-vented bulbuls to long and short photoperiods also resemble light responses of birds breeding at temperate latitudes during spring and/or early summer months (see reviews: Farner and Follett, 1966; Lofts and Murton, 1968; Farner and Lewis, 1971; Murton and Westwood, 1977; Assenmacher and Jallageas, 1978, 1980; Wingfield, 1983; Sharp, 1984a,b; Nicholls *et al*, 1988; Deviche and Small, 2002; Dawson, 2008, 2015; Nakane and Yoshimura, 2019).

To our knowledge, till date, there is no report on light response of red-vented bulbuls from other latitudes of its current distribution ranges (30^{o}N-36^{o}S) to make comparisons between light responses of birds from different latitudes. Further, it is logical to surmise that because of small seasonal changes in natural lighting hours in the tropics and subtropics, daylength might not serve as dominant environmental cue for timing seasonality in birds (Lofts and Murton, 1968; Farner and Lewis, 1971). Though some birds have been shown to exhibit photoperiodic control over their different physiological attributes, it is difficult to discern the photoperiodic acumen of birds of these regions because only few of them have been examined experimentally. At lower latitudes, some resident forms like Indian weaver bird, *Ploceus philippinus* (Thapliyal and Saxena, 1964), wood pigeon, *Columba palumbus* (Lofts *et al*, 1967a), rufous collared sparrow, *Zonotrichia capensis hypoleuca* (Lewis *et al*, 1974) and red-cross bill, *Loxia curvirostra* (MacDougall-Shackleton *et al*, 2006) have been noticed to respond to stimulatory and/or inhibitory daylengths in much traditional ways as their gonads remain small and inactive when birds are on short days and exposure to long days prompts the development of gonads which are sustained so long birds are maintained on long days. Obviously, in such birds, daylength serves as the key driving force and birds lack a photorefractory state in their sexual cycle. In such species, seasonality is maintained by waxing and waning of daylengths. In Japanese quail, *Coturnix coturnix japonica*, gonads remain developed under long days but decline rapidly owing to small decreases in lighting hours despite the prevailing daylength being longer than which had initially resulted their growth earlier in the season. Such birds are referred to be relatively refractory as their gonads redevelop under long daylengths and remain maintained so long birds are kept on long days (Robinson and Follett, 1982; Follett and Pearce-Kelly, 1990). However, other sedentary forms respond to daylengths quite differently. For instance, gonads of black-headed munia, *Munia malacca malacca,* develop and remain large under daylengths ranging from 8-24 hours (Pandha and Thapliyal, 1969). Gonads of spotted munia, *Lonchura punctulata* enlarge under ultra short days (0.25-6hrs) but customary short and long photoperiods (8-24hr) fail to induce gonadal recrudescence, instead long days tend to inhibit or retard gonadal growth (Chandola *et al*, 1975). In lal munia, *Estrilda amandava*, short daylengths have no effects but long days induce recurrent development and regression of testes at the individual level (Lal and Thapliyal, 1995). Red-billed quelea, *Quelea quelea* responds to changes in photoperiod and birds experience a brief refractory period in their sexual cycle which terminates spontaneously irrespective of daylengths they are maintained at (Lofts, 1962). Present study in red-vented bulbul, *Pycnonotus cafer stanfordi* is in sharp contrast with avian models described above but has great resemblance with light responses of some other sub-tropical species like red-headed buntings, *Emberiza bruniceps* (Lal and Thapliyal, 1985a), black-headed buntings, *Emberiza melanocephala* (Devi and Lal, 1994), rose finch, *Carpodacus erythrinus* (Tewary and Dixit, 1983), Yellow-throated sparrows, *Gymnorhis xanthocollis* (Tewary and Dixit, 1986), house sparrows, *Passer domesticus* (Trivedi *et al*, 2006) and tree sparrows, *Passer montanus* (Dixit and Singh, 2011, 2012).

Incomplete development of gonads in birds kept on constant 12L/12D photoperiod may be due to sub-maximal release of GnRH-I and/or gonadotrophins from the hypothalamo-hypophyseal complex. Daily lighting durations in excess of 12hr are also necessary for gonadal enlargement in red-headed bunting, *Emberiza bruniceps* (Prasad, 1983), rose finch, *Corpodacus erythrinus* (Tewary and Dixit, 1983) and yellow-throated sparrow, *Gymnorhis xanthocollis* (Tewary and Dixit, 1986). Further, partially developed gonads of bulbuls under 12L/12D photoperiod were sustained for at least 07 months and during this period presumptive moult was also arrested. It remains to be established whether retention of bulbuls on 12L/12D for longer durations would manifest in gonadal fluctuations to maintain annual cyclic rhythms as reported in the male starlings, *Sturnus vulgaris* under 12L/12D photoperiodic regimen (Schwab, 1971). In many species, light has been shown to act as a zeitgeber to entrain endogenous circannual rhythms in physiological and behavioural attributes under seasonally constant environmental conditions (Assenmacher, 1974; Farner and Gwinner, 1980; Gwinner, 1981). Present response of red-vented bulbuls to 12L/12D daylength is however, at variance with photoperiodic response of tree sparrows which share habitat with red-vented bulbuls at the study site in Lumami. Gonads of tree sparrows develop fully under fixed duration of 11 L/13D followed by regression and moult at par with birds maintained on constant 14L/10D photoperiod (Neelakshi, unpublished; Dixit and Singh, 2011).

The development and regression patterns of gonads in birds transferred from NDL to constant 16L/8D and 20L/4D daylengths from December–May, presented the most intriguing feature as gonads developed like temperate-zone birds under both the photoperiodic regime but their development rates were not significantly different from each other and gonadal peaks appearing 03 (December and January groups), 02 (February–April groups) and 01 (May group) month after exposures to light did not differ significantly. Further, unlike birds breeding at higher latitudes slow post-nuptial gonadal regression ensued in bulbuls. This feature is unique in red-vented bulbuls which contrasts photoperiodic responses of birds examined at temperate latitudes in which gonadal development and regression rates had positive correlation with daily durations of light (Nicholls *et al*, 1988; Dawson, 2015; Nakane and Yoshimura, 2019). In the present case, it is interesting to note that photostimulation during December led to about 60% enlargement of gonads as compared to maximum gonadal size of NDL birds in June. Whether this shows incomplete resumption of photosensitivity in captive birds or poor sensitivity of gonads to pituitary gonadotrophins during February-March, or even the absence of some natural factors which, when present, help the bird to attain maximum gonadal size, is not clear. Such a supposition however, gains no support when viewed together with full testicular enlargement in birds shifted to constant 16L/8D and 20L/4D daylengths from January – March periods. Further, lack of an impact of photostimulation on testicular development during April and May months probably denote that in the red-vented bulbuls gonadal response to long daylengths depends upon the physiological status of birds at the time of photostimulation or alternatively, the neuroendocrine-gonadal axis exhibits seasonal changes in the sensitivity to photoperiodic stimulation. There are reports of seasonal cycle of photosensitivity in gonads of weaver bird, *Ploceus philippinus* (Singh and Chandola, 1981), red-headed bunting, *Emberiza bruniceps* (Lal and Thapliyal, 1985a) and tree sparrow, *Passer montanus* (Dixit and Singh, 2012). It is also known that in red-vented bulbuls egg-deposition occurs following monsoon rains (see Chapter-I).

Further, the observation that gonads of birds developed under constant 16L/8D and 20L/4D daylengths regressed on prolonged exposures to light owing probably to the development of photorefractoriness. This response could also be seen expressing in NDL birds during post-breeding period when fully developed (June) and/or regressing (August) testes of birds decreased normally following transfer to constant 16L/8D or 20L/4D daylengths. Further, decrease in developed and/or regressing testes of red-vented bulbuls following exposure to constant 15L/9D daylength on par with NDL birds has also been reported from Varanasi (25^{o}N) (Lal and Thapliyal, 1982a). The facts that regressed testes of NDL birds in September failed to develop following transfer to 16L/8D or 20L/4D photoschedules and regressed testes under 16L/8D remained unaffected when birds were moved to continuous illumination, denote that red-vented bulbuls exhibit 'absolute' refractoriness during post-nuptial regression of gonads. This response of red-vented bulbuls is common with subtropical birds like red-headed buntings, *Emberiza bruniceps* (Thapliyal and Lal, 1984a), brahminy myna, *Sturnia pagodarum* (Kumar and Kumar, 1991), black-headed buntings, *Emberiza melanocephala* (Devi and Lal, 1994), and Eurasian tree sparrows, *Passer montanus* (Dixit and Singh, 2011, 2012) maintained on simulated long daylengths. In these species examined at sub-tropical latitudes, post-breeding gonadal regression is reported to manifest as a result of development of absolute photorefractoriness.

Significant testicular development in 60% birds transferred from NDL to constant 16L/8D and 20L/4D daylengths during October and the response increasing to 80% when birds were photostimulated in November and the magnitude of testicular enlargement in these birds being significantly more as compared to October groups, is suggestive of progressive transition of light responding mechanisms from photorefractoriness to photosensitivity beginning around autumnal equinox and progressing with naturally decreasing lighting hours towards winter solstice. Rapid gonadal growth with maximum enlargement of testes and ovarian follicles in 3rd month followed by regression in birds shifted to 16L/8D and 20L/4D daylengths during December advocates in favour of progressive dissipation of photorefractoriness with natural decreases in daylengths during autumn and winter months. Further, not all the birds emerged from photorefractoriness at one go. This response of red-vented bulbuls contradicts findings in slate coloured junco, *Junco hyemalis*, white-throated sparrow, *Zonotrichia albicollis* and white-crowned sparrow, *Zonotrichia leucophrys gambelii*, in which photorefractoriness terminates abruptly in a single step (Shank, 1959; King *et al*, 1960). Progressive increase in photosensitivity during autumn and winter months has also been reported in red-headed bunting, *Emberiza bruniceps* (Lal and Thapliyal, 1985a) and tree sparrow, *Passer montanus* (Dixit and Singh, 2012).

Seasonal fluctuations in body mass of NDL birds with maxima coinciding with gonadal peak and post-nuptial decline of body mass concomitant with gonadal regression during June-July may denote that concurrent increase and decrease in body mass and gonads of red-vented bulbuls have causal relationship and may be coordinated by some common physical or factors. It is likely that seasonal increases in daylengths after winter solstice might influence body mass regulating mechanisms to gear up metabolic preparations in advance of breeding season as shown in sub-tropical birds like red-headed and black-headed buntings (Thapliyal and Lal, 1984a; Lal and Thapliyal, 1985a; Lal, 1988; Devi and Lal, 1994). This tendency has also featured in bulbuls shifted from NDL to constant 16L/8D and 20L/4D photoperiods from December to April periods wherein maximum body mass appeared 02 (December-January) and 01 (February-April) month after photostimulation preceding gonadal peak. It may not therefore, be unreasonable to deduce that low body mass of birds exposed to constant 8L/16D during December might result due to lack of critical daylength necessary for sustaining body mass progression as observed in NDL birds during April-May period. Absence of significant fluctuations in body mass of bulbuls exposed to constant 12L/12D photoperiod during December strengthens this view. Present results however, disagree with general assumption that residential birds do not deposit energy resources to add significance differences in body mass (Farner and Follett, 1966), instead support findings reported in many Indian finches which economize their energy resources to support calorie requirements towards successful rearing of the young ones and thus better survival prospects (Thapliyal, 1981, 1992). It is noticeable that body mass of NDL birds declined simultaneously with regressing gonads from June to September. Furthermore, during this period, shifting of birds from NDL to 16L/8D or 20L/4D failed in preventing further decline in body mass or induced an increase in their body weight. This might denote that during this period birds were metabolically refractory. This observation is consistent with the finding that photoinduced increase in body mass of birds was followed by decline which remained low so long birds were maintained on long days. Further, the observation that transfer of such birds from 16L/8D to continuous illumination failed in evoking a second or additional increase in body mass denotes that similar to gonads, body mass regulating mechanisms exhibit absolute metabolic refractoriness during annual body mass cycle. Post-breeding metabolic refractoriness has also been reported in many other avian species which deposit moderate to huge calorie resources to cope with energy requirements towards migration, moult and reproduction (see reviews: Farner and Follett, 1966; Farner and Lewis, 1971; Nicholls *et al*, 1988; Thapliyal and Gupta, 1989; Thapliyal, 1992). It is significant to note that decrease in body mass of NDL birds from June to September occurred due to rapid calorie drainage during intense breeding activities and rearing of young when long daylengths were not able to influence body mass regulating mechanisms. Resurgent increase in body mass of NDL birds during October-November may thus be accounted to the dissipation of metabolic refractoriness and consequent increase in body mass.

Onset of PFs and BFs in both the sexes of bulbuls in June followed by rapid increase to peak in August and followed by decline shows that feather moult in red-vented bulbul is linked to decreasing levels of sex steroids in the circulation. Initiation of moult coinciding gonadal regression and consequent decrease in circulatory levels of gonadal steroids have also been reported in several species breeding at sub-tropical and temperate latitudes (Svensson and Nilson, 1997; Dixit and Singh, 2011, 2012). Inhibition of moult in both, male and female birds held on constant 12L/12D and 8L/16D photoperiods probably point to the fact that the process of moult is linked to post-nuptial decline in sex steroids in the circulation. It is however, not clear whether moulting manifests due to direct influence of daylength or it results as a secondary impact owing to dipping plasma levels of gonadal steroids. It is however, probable that these two events are causally related and their intimate linkages cater to match ecological requirements of a species and their congeners to accommodate vitally important physiological events like migration, reproduction and moult to precipitate coinciding presence of surplus food resources in the habitat. There is evidence to show that as in many other species (Wong, 1983; Dixit and Singh, 2011, 2012) though within a narrow limit, breeding and moult differ in red-vented bulbuls in different eco-habitats (Prajapati *et al*, 2011; Sharma and Sharma, 2013; Rao *et al*, 2013; Zia *et al*, 2013; Awais *et al*, 2014; Zohaib *et al*, 2021).

Viewing the overall scenario of light responses of red-vented bulbuls, *Pycnonotus cafer stanfordi* from the perspective of its practical uses in timing the breeding season, it may be surmised that daily photoperiod around 11hrs may be critical for booting neuroendocrine mechanisms prompting initiation of gonadal growth and improvement of body reserves. These indices progress further as daylengths advance, attain peak around summer solstice and then decrease owing to the development of absolute refractoriness -gonadal and metabolic- paving way to timely renewal of feathers. Photorefractoriness is dissipated naturally by decreasing daylengths between autumnal equinox and the winter solstice. Thus, at 26^{o}N, under normal circumstances, neuroendocrine mechanisms of red-vented bulbuls are tuned to operate within the catch of 10.5-13.5hr from winter solstice to summer solstice. Daylengths outside this spectrum specially on the higher sides cast negligible impact on the development and/or regression of gonads, albeit post-breeding gonadal regression ensue faster in birds exposed to constant 16L/8D and 20L/4D during April and May as compared to NDL birds. This being the pertinent feature would result shrinkage of breeding periodicity at higher latitudes and thus may not be to the obvious advantage of the species to extend their dwellings towards temperate latitudes. Throughout their current native ranges red-vented bulbul populations have been noticed to have extended breeding season to start around spring equinox and drawn up to autumnal equinox (McCann, 1932; Dutt, 1932; Lamba, 1968; Vijayan, 1980; Bhatt and Kumar, 2001; Balkrishnan, 2010; Prajapati *et al*, 2011; Rao *et al*, 2013; Awais *et al*, 2014; Thiabault *et al*, 2018, 2019, Zohaib *et al*, 2021). Reportedly, red-vented bulbuls are very aggressive and inherit a strong tendency of expanding their occupational territory (Thibault *et al*, 2019). Further, the distribution spectrum of *Pycnonotus cafer stanfordi*, is limited to the North-East India (southern Assam, Nagaland, Arunachal Pradesh, Manipur, Meghalaya, Tripura), Bangladesh, northern Myanmar and south-western China between 23^{o}N-29^{o}N (ZSI, 2006) within a narrow spectrum of 13-14 hr/day with longest daylength at the summer solstice. It is therefore, not unreasonable to suggest that photoperiodic mechanism of red-vented bulbuls is naturally programmed to operate optimally within photoperiodic spectrum of 10-14 hr/day and daylengths outside this spectrum especially on higher sides would shorten reproductive periodicities. Relatively rigid photoperiodic response system in red-vented bulbul seems to be highly adaptive since it has credential to help determining latitudinal distribution of the sub-species.

Table-10 : Effect of simulated Long and Short Daylengths on Testicular Cycles of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami (26^{o}N).*

Months *2018*	NDL	12L/12D	16L/8D	20L/4D	8L/16D
	Testis Size *(mm^{3}±SE)*	Testis Size *(mm^{3}±SE)*	Testis Size *(mm^{3}±SE)*	Testis Size *(mm^{3}±SE)*	Testis Size *(mm^{3}±SE)*
December	0.93±0.04^{c}	0.78±0.79^{c}	0.90±0.05^{c}	0.86±0.04^{c}	0.86±0.04
January	1.16±0.05^{c}	8.33±3.10^{a}	22.55±6.53^{b}	26.27±6.48^{c}	0.90±0.05
February	5.20±0.50^{c}	15.59±1.92	45.62±7.88^{a}	46.47±10.97^{b}	0.82±0.07
March	12.01±2.74^{c}	21.93±4.04	63.05±7.05	69.94±7.88	0.90±0.05
April	25.78±5.14^{c}	26.57±4.91	46.14±1.86^{b}	44.91±7.83^{b}	0.90±0.05
May	74.66±6.87^{a}	23.43±6.58	26.18±5.01^{c}	17.68±0.53^{c}	0.86±0.04
June	108.16±10.70	23.11±3.84	11.79±2.48^{c}	5.29±1.73^{c}	0.83±0.04
July	56.33±9.65^{b}	26.18±5.05	3.98±0.74^{c}	1.84±0.33^{c}	0.79±0.00
August	21.70±2.95^{c}	25.86±4.50	1.61±0.34^{c}	1.05±0.26^{c}	0.79±0.00
September	8.83±0.81^{c}	23.11±3.84	0.86±0.04^{c}	0.86±0.04^{c}	0.86±0.04

*****Values are Mean ± *SEM*, n=6 in each group.

NDL: F$_{(9,50)}$=21.41, p<0.0001; 12L12D: F$_{(9,50)=}$ 4.52, p<0.001; 16L/8D: F$_{(9,50)}$=5.67, p<0.0001; 20L/4D: F$_{(9,50)}$=59.66, P<0.0001; 8L/16D: F$_{(9,50)}$= 0.42, (based on One Way Independent Measure of ANOVA).

^{a,b,c} differ from the highest value within group at p<0.05; <0.01 & <0.001 level respectively (Student's '*t*' test).

Table- 11: Effect of Simulated Long and Short Daylengths on Body Mass of Male Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami (26^{o}N).*

Months *2018*	NDL	12L/12D	16L/8D	20L/4D	8L/16D
	Body Mass *(g±SE)*	Body Mass *(g±SE)*	Body Mass *(g±SE)*	Body Mass *(g±SE)*	Body Mass *(g±SE*
December	37.20±0.76	38.53±0.59	38.77±0.53	37.95±0.85	38.60±0.74
January	37.60±0.97	38.72±0.47	39.18±0.33	39.00±0.63	37.70±0.58
February	37.42±0.73	39.42±0.57	39.42±0.62	39.42±0.50	37.05±0.42
March	37.65±0.55	39.17±0.46	38.47±0.91	38.52±0.80	36.02±0.38
April	38.28±0.42	29.28±0.39	36.62±0.48[a]	36.23±0.38[a]	35.72±0.31[a]
May	39.01±0.37	39.10±0.55	35.62±0.32[b]	35.28±0.35[b]	35.43±0.30[b]
June	38.08±0.37	39.08±0.51	35.00±0.26[c]	34.85±0.25[c]	34.87±0.23[b]
July	37.25±0.47[b]	38.73±0.35	34.78±0.36[b]	34.45±0.32[c]	34.73±0.26[b]
August	35.38±0.46[c]	39.08±0.38	34.92±0.33[c]	34.78±0.35[c]	34.93±0.48[b]
September	36.00±0.39[c]	38.53±0.59	34.60±0.31[c]	34.78±0.18[c]	35.65±0.30[b]

*****Values are Mean ± *SEM*, n=6 in each group.
NDL: $F_{(9,50)}$=4.81, p<0.01; 12L12D: $F_{(9,50)}$= 0.47; 16L/8D: $F_{(9,50)}$=3.30, p<0.05; 20L/4D: $F_{(9,50)}$=3.1, P<0.05; 8L/16D : $F_{(9,50)}$= 3.42, p<0.0001 (based on One Way Independent Measure of ANOVA).

[a,b,c] differ from the highest value within the group at p<0.05; <0.01 & <0.001 level respectively (Student's '*t*' test).

Table- 12 : Effect of Simulated Long and Short Daylengths on Follicular Cycle of Red-vented Bulbul, *Pycnonotus cafer Stanfordi* Deignan at Lumami (26°N).*

Months *2018*	8L/16D	12L/12D	16L/8D	20L/4D	NDL
	Follicle Size *(mm±SE)*	Follicle Size *(mm±SE)*	FollicleSize *(mm±SE)*	Follicle Size *(mm±SE)*	Follicle Size *(mm±SE)*
December	0.25	0.25	0.25	0.25	0.25
January	0.25	0.25	0.75±0.12[a]	0.92±0.15[a]	0.25
February	0.25	0.25	1.18±0.15	1.20±0.13[a]	0.25
March	0.25	0.50±0.00	1.60±0.15	1.75±0.10	0.25
April	0.25	0.67±0.11	0.87±0.11[b]	0.58±0.08[b]	0.67±0.11[b]
May	0.25	0.58±0.08	0.50±0.00	0.50±0.00	1.38±0.20[a]
June	0.25	0.50±0.00	0.25±0.00	0.25±0.00	2.60±0.22
July	0.25	0.50±0.00	0.25±0.00	0.25±0.00	0.98±0.08[c]
August	0.25	0.50±0.00	0.25±0.00	0.25±0.00	0.50±0.00[c]
September	0.25	0.50±0.00	0.25±0.00	0.25±0.00	0.25±0.00

*Values are Mean±*SEM*, n= 6 in each group.

NDL: $F_{(9,50)}$ 24.93, p<0.0001; 12L12D: $F_{(9,50)}$= 5.33, p<0.0001; 16L/8D: $F_{(9,50)}$=23.96, p<0.0001; 20L/4D: $F_{(9,50)}$=24.15, P<0.0001; 8L/16D: $F_{(9,50)}$= 0.14, (based on One way Independent Measures of ANOVA).

[a,b,c] differ from the highest value within the group at p<0.05; <0.01 & <0.001 level respectively (Student's '*t*' test).

Table- 13: Effect of Simulated Long and Short Daylengths on Body Mass of Female Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami (26^{o}N)**.***

Months *2018*	8L/16D	12L/12D	16L/8D	20L/4D	NDL
	Body Mass *(g±SE)*	Body Mass *(g±SE)*	Body Mass *(g±SE)*	Body Mass *(g±SE)*	Body Mass *(g±SE*
December	37.57±0.44	38.53±0.59	38.37±0.53	37.00±0.85	36.82±0.48
January	36.85±0.58	38.72±0.47	37.68±0.67	37.97±0.80	36.83±0.54
February	36.40±0.23	39.42±0.57	38.43±0.54	38.48±0.61	37.32±0.47
March	36.38±0.39	39.17±0.46	38.02±0.63	37.72±0.58	37.33±0.53
April	35.78±0.32^{a}	39.28±0.39	37.23±0.56	37.77±0.50	37.38±0.47
May	35.42±0.21^{a}	39.10±0.55	35.87±0.43^{a}	35.50±0.29	38.88±0.47
June	35.17±0.21^{c}	39.08±0.51	35.38±0.31^{a}	34,75±0.25^{a}	36.62±0.35
July	35.17±0.17^{c}	38.73±0.35	34.68±0.21^{b}	34.82±0.42^{a}	36.37±0.30^{a}
August	35.28±0.35^{c}	39.08±0.38	34.50±0.29^{b}	34.48±0.43^{b}	35.90±0.37^{b}
September	35.65±0.30^{b}	38.53±0.59	34.60±0.31^{b}	34.78±0.18^{b}	36.00±0.39^{a}

*Values are Mean±*SEM*, n= 6 in each group.

NDL: $F_{(9,50)}$=3.57, p<0.001; 12L12D: $F_{(9,50)}$= 0.36,; 16L/8D: $F_{(9,50)}$=3.14, p<0.01; 20L/4D: $F_{(9,50)}$=2.68, P<0.01; 8L/16D: $F_{(9,50)}$= 3.42, p<0.01 (based on One Way Independent Measure of ANOVA).

^{a,b,c} differ from the highest value within the group at p<0.05; <0.01 & <0.001 level respectively (Student's '*t*' test).

Table-14 : Effect of simulated Long and Short Daylengths on Feather Moult of Male Red-Vented Bulbul, *Pycnonotus cafer Stanfordi* Deignan at Lumami (26^{o}N).*

Months *2018*	NDL		12L/12D		16L/8D		20L/4D		8L/16D	
	PFs	BFs	PFs	BFs	PFs	BFs	PFs	BFs	PFs	BFs
December										
January										
February										
March										
April			-	-	5.23±0.94^{c}	3.44±0.62^{a}	7.53±1.57^{c}	1.98±0.32^{b}	-	-
May			-	-	29.00±.5.46	5.55±0.64	19.92±3.60^{c}	4.85±0.94^{a}	-	-
June	1.33±0.89^{c}	1.13±0.53^{a}	-	-	30.00±4.00	7.50±0.56	33.01±3.50	9.21±1.65	-	-
July	22.70±.3.56	6.43±1.24	-	-	23.53±3.12	4.17±0.79^{b}	27.41±4.21	3.67±0.89^{b}	-	-
August	27.86±3.25	8.30±1.36	-	-	6.73±1.23^{c}	2.42±0.4^{c}	9.57±1.62^{c}	1.42±0.36^{c}	-	-
September	20.33±3.453	3.29±0.63^{b}	-	-	2.41±0.63^{c}	1.08±0.19^{c}	0.53±0.04^{c}	0.81±0.17^{c}	-	-

*Values are Mean±*SEM*, n= 6 in each group.

^{a,b,c} differ from the highest value within the group at p<0.05; <0.01 & <0.001 level respectively (Student's '*t*' test).

Table-15: Effect of Simulated Long and Short Daylengths on Feather Moult of Female Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami (26°N).*

Months *2018*	NDL		12L/12D		16L/8D		20L/4D		8L/16D	
	PFs	BFs	PFs	BFs	PFs	BFs	PFs	BFs	PFs	BFs
December										
January										
February										
March										
April			-	-	5.46±1.27[c]	3.13±1.11[b]	4.83±0.87[c]	3.10±0.53[b]	-	-
May			-	-	18.37±.3.14	4.55±0.64	18.78±3.46[a]	6.12±1.34	-	-
June	2.48±1.32[c]	1.17±0.54[c]	-	-	26.80±5.36	7.60±1.23	29.88±4.20	6.50±0.56	-	-
July	23.50±3.43[a]	6.35±0.54	-	-	20.23±3.23	3.17±0.83[b]	11.33±3.43[c]	3.17±0.79[b]	-	-
August	32.03±3.22	6.50±0.46	-	-	6.50±1.32[c]	1.32±0.36[c]	3.50±1.63[c]	1.42±0.46[c]	-	-
September	17.65±4.23[b]	3.17±0.54[c]	-	-	0.83±0.64[c]	0.60±0.19[c]	0.63±0.40[c]	0.58±0.19[c]	-	-

*Values are Mean±SEM, n= 6 in each group.

[a,b,c] differ from the highest value within the group at p<0.05; <0.01 & <0.001 level respectively (Student's '*t*' test).

Table- 16: Effect of Constant 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented Bulbul *Pycnonotus cafer stanfordi* Deignan.*

Months *2018*	16L/8D		20L/4D		NDL	
	Body Mass *(g±SE)*	Testicular Size *(mm3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm3±SE)*	Body Mass (g±SE)	Testicular Size *(mm3±SE)*
January	37.66±0.56	2.08±0.42^{c}	37.42±0.42	1.34±0.39^{c}	37.32±0.56	1.41±1.06^{c}
February	37.76±0.65	55.08±7.72^{c}	37.88±0.60	67.34±10.69^{c}	37.62±1.06	3.41±1.06^{c}
March	38.53±0.64	63.34±8.95	38.35±0.49	78.22±8.40	37.74±0.89	11.26±2.65^{c}
April	38.18±0.58	76.82±8.22	38.00±0.43	80.56±9.83	38.04±0.73	27.67±5.86^{c}
May	36.46±0.53	39.58±8.15^{a}	36.37±0.37	42.74±5.66^{b}	38.56±0.81	73.87±8.35^{a}
June	35.84±0.31^{a}	13.36±2.91^{c}	35.72±0.33^{a}	18.13±3.16^{c}	37.38±0.69	105.18±11.19
July	35.62±0.24^{b}	5.35±1.23^{c}	35.24±0.46^{b}	5.83±0.72^{c}	37.16±0.67	54.68±9.57^{b}
August	35.48±0.21^{b}	2.11±0.31^{c}	35.18±0.25^{b}	1.87±0.15^{c}	36.32±0.51^{a}	30.64±2.90^{c}

*Values are Mean±*SEM*, n= 5 in each group.

^{a,b,c} differ from the highest value of indices within the group at $p<0.05$; <0.01 & <0.001 level respectively (Student's '*t*' test).

Table- 17: Effect of constant 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented Bulbul *Pycnonotus cafer stanfordi* Deignan.*

Months *2018*	16L/8D		20L/4D		NDL	
	Body Mass *(g±SE)*	Testicular Size *(mm3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm3±SE)*	Body Mass (g±SE)	Testicular Size *(mm3±SE)*
February	37.86±0.67	5.08±0.72^{c}	38.38±0.53	7.34±13.69^{c}	37.92±1.06	3.41±1.06^{c}
March	38.68±0.56	63.94±8.95^{a}	39.06±0.59	68.22±9.40^{b}	37.74±0.89	11.26±2.65^{c}
April	38.38±0.62	86.42±8.21	39.00±0.42	102.56±12.23	38.04±0.73	27.67±5.86^{c}
May	36.36±0.33	39.58±11.15^{b}	36.82±0.17	42.74±5.66^{c}	38.56±0.81	73.87±8.35
June	35.94±0.20	23.36±2.91^{c}	36.02±0.29	21.11±3.16^{c}	38.08±0.99	105.18±11.19
July	35.82±0.20^{b}	13.35±1.23^{c}	35.54±0.26^{b}	12.83±0.92^{c}	37.16±0.67	54.68±9.57^{b}
August	35.78±0.25^{b}	1.15±0.31^{c}	35.36±0.25^{b}	0.87±0.05^{c}	36.32±0.51^{a}	30.64±2.90^{c}

*Values are Mean±*SEM*, n= 5 in each group.

^{a,b,c} differ from the highest value within the group at $p<0.05$; <0.01 & <0.001 level respectively (Student's '*t*' test).

Table-18. Effect of constant 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented Bulbul *Pycnonotus cafer stanfordi* Deignan.*

Months *2018*	16L/8D		20L/4D		NDL	
	Body Mass *(g±SE)*	Testicular Size *(mm3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm3±SE)*	Body Mass (g±SE)	Testicular Size *(mm3±SE)*
March	37.38±0.56	13.21±3.25^{c}	37.56±0.46	8.22±2.40^{c}	37.44±0.52	11.26±1.65^{c}
April	37.88±0.42	73.30±9.21	38.00±0.42	82.56±11.29	38.04±0.73	27.67±5.86^{c}
May	36.36±0.33	98.58±10.15	37.02±0.37	110.64±9.26	38.56±0.81	73.87±8.35
June	35.94±0.36	43.31±10.11^{b}	36.02±0.29	41.11±8.44^{b}	38.08±0.99	105.18±11.19
July	35.75±0.40^{b}	18.35±6.22^{c}	35.32±0.37^{b}	13.65±2.83^{c}	37.16±0.67	54.68±9.57^{b}
August	35.13±0.41^{b}	1.15±0.31^{c}	35.36±0.25^{b}	0.87±0.05^{c}	36.32±0.51^{a}	30.64±2.90^{c}

*Values are Mean±*SEM*, n= 5 in each group.

^{a,b,c} differ from the highest value within the group at $p<0.05$; <0.01 & <0.001 level respectively (Student's '*t*' test).

Table- 19 : Effect of constant 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan*

Months *2018*	16L/8D		20L/4D		NDL	
	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*
April	38.54±0.82	29.77±6.80^{c}	38.48±0.74	31.88±5.36^{c}	38.86±0.53	28.11±5.66^{c}
May	39.14±0.45	70.83±4.79^{b}	38.70±0.52	86.22±13.26	38.74±0.53	73.87±8.35^{a}
June	37.46±0.34	115.86±9.86	37.84±0.46	119.52±11.83	37.48±0.46	105.18±11.19
July	36.68±0.29^{b}	13.83±3.07^{c}	36.88±0.58^{a}	22.54±4.88^{c}	36.70±0.38^{a}	51.78±6.86^{c}
August	36.30±0.38^{c}	2.83±0.91^{c}	35.78±0.36^{b}	2.05±0.31^{c}	35.88±0.29^{b}	11.32±3.07^{c}

*Values are Mean±SE, n= 5 in each group.

^{a,b,c} differ from the highest value within the group at $p<0.05$; <0.01 and <0.001 level respectively (Student's '*t*' test).

Table- 20 : Effect of constant 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan*

Months *2018*	16L/8D		20L/4D		NDL	
	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*
May	38.64±0.65	70.83±4.79^{b}	38.60±0.52	76.22±11.34	38.74±0.53	73.87±8.35^{a}
June	37.46±0.34	118.12±9.86	37.54±0.54	110.32±10.24	37.48±0.46	105.18±11.19
July	36.68±0.29^{a}	13.83±3.07^{c}	36.38±0.65^{a}	22.54±4.88^{c}	36.70±0.38^{a}	51.78±6.86^{c}
August	36.30±0.38^{c}	2.83±0.91^{c}	35.57±0.47^{b}	2.05±0.31^{c}	35.88±0.29^{b}	11.32±3.07^{c}
September	35.21±0.48^{b}	1.20±0.30^{c}	35.21±0.53^{c}	1.02±0.11^{c}	35.42±0.24^{c}	2.29±1.03^{c}

*Values are Mean±SE, n= 5 in each group.

^{a,b,c} differ from the highest value within the group at $p<0.05$; <0.01 & <0.001 level respectively (Student's '*t*' test).

Table- 21: Effect of constant 16L/8D photoperiod on Testicular size and Body Mass of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan*

Months *2018*	16L/8D		20L/4D		NDL	
	Body Mass *(g±SE)*	Testicular Size *(mm3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*
June	37.79±0.44	108.84±13.69	37.42±0.57	108.84±13.69	38.06±0.37	111.45±12.44
July	36.22±0.32	28.11±5.66^c	36.18±0.47	13.36±2.91^c	36.82±0.31	38.10±5.21^c
August	35.40±0.25^a	1.77±0.36^c	35.40±0.28	1.54±0.33^c	35.78±0.25	8.80±3.22^c
September	35.40±0.25^a	0.92±0.01^c	35.14±0.19^a	0.92±0.01^c	35.42±0.24^a	2.29±1.03^c

*Values are Mean±*SEM*; n=5 in each group.

^{a,b,c} differ from the value of June at $p<0.05$;<0.01 and <0.001 level respectively (Student's '*t*' test).

Table-22: Effect of constant 16L/8D photoperiod on Testicular size and Body Mass of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan*

Months *2018*	16L/8D		20L/4D		NDL	
	Body Mass *(g±SE)*	Testicular Size *(mm3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*
July	36.80±0.54	43.24±8.36	36.46±0.54	36.36±5.84	35.97±0.63	38.10±5.21
August	35.68±0.45	11.12±3.88^c	35.22±0.43	9.87±3.24^c	35.68±0.54	8.80±3.22^c
September	35.20±0.35	1.92±0.42^c	35.35±0.39	1.82±0.21^c	36.42±0.24	2.29±1.03^c

*Values are Mean±*SEM*; n=5 in each group.

^{c}differs from the value of July within the group at p<0. 001 level (Student’s ‘*t*’ test).

Table-23 : Effect of Simulated Long Daylengths on Testicular size and Body Mass of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan.*

Months *2018*	16L/8D		20L/4D		NDL	
	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*
August	36.54±0.35	6.87±2.05	36.30±0.37	7.92±2.15	36.38±0.46	8.87±3.05
September	35.94±0.20	1.83±0.24[a]	35.58±0.25	2.03±0.34	36.58±0.57	1.87±0.25[a]
October	35.34±0.17[e]	0.79±0.00[c]	35.26±0.24[d]	0.83±0.04[a]	37.36±0.58	0.83±0.04[a]

*Values are Mean±*SEM*; n=5 in each group.

[a,c] differ from within group testicular size in August at $p<0.05$ and $p<0.001$ level respectively (Student's '*t*' test).

[d,e] differ from within group body mass in August at $p<0.05$ and $p<0.001$ respectively (Student's '*t*' test).

Table-24: Effect of Simulated Long Daylengths on Testicular size and Body Mass of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan.*

Months *2018*	16L/8D		20L/4D		NDL	
	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*
September	36.94±0.56	1.58±0.45	36.50±0.48	1.62±0.45	36.68±0.46	1.46±0.55
October	35.84±0.50^a	1.03±0.36	35.68±0.36^a	1.03±0.34	37.52±0.49	1.07±0.25
November	35.46±0.47^a	0.79±0.32	35.35±0.36^a	0.83±0.04	37.86±0.58	0.83±0.04

*Values are Mean±*SEM*; n=5 in each group. ^{a}differs from body mass of NDL group in corresponding months at $p<0.05$ (Student' 't' test.

Table-25: Effect of Simulated 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan.*

Months 2018	16L/8D		20L/4D		NDL	
	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*
October	37.24±0.46	0.83±0.4	36.80±0.21	0.83±0.04	37.62±0.40	0.79±0.00
November	37.38±0.56	2.60±0.67[a]	36.76±0.20	3.61±1.13[a]	38.24±0.47	0.79±0.00
December	36.34±0.54	1.87±0.35[a]	35.98±0.16	1.39±0.40[a]	38.42±0.39	0.79±0.00
January	35.32±0.38[a]	0.79±0.79	35.32±0.10[a]	0.79±0.00	38.02±0.53	0.83±0.04

*Values are Mean±SE; n=5 in each group.

[a] differs from the value of NDL birds in corresponding months at p<0.05 level (Student’s ‘*t*’ test).

Table-26 : Effect of Simulated Long days on Testicular size and Body Mass of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan.*

Months *2018*	16L/8D		20L/4D		NDL	
	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*
November	38.10±0.45	0.87±0.05	37.46±0.62	0.83±0.04	37.98±0.45	0.79±0.00
December	38.00±0.44	16.34±2.62^c	37.20±0.48	17.20±2.91^c	38.28±0.27	0.83±0.04
January	36.00±0.38^d	10.72±3.96^a	35.20±0.28^d	14.34±3.40^a	37.68±0.71	0.87±0.05
February	35.22±0.30^d	3.28±0.40	34.56±0.19^d	5.48±0.12	37.48±0.44	4.36±1.12^e

*Values are Mean±*SEM*; n=5 in each group.

^{a,c}differ from the value of NDL birds in corresponding months at $p<0.05$ and $p<0.001$ level respectively (Student's '*t*' test).
^{e}differs from within group value of testes of NDL birds in February at $p<0.05$ level (Student's '*t*' test).
^{d}differs from the mean body mass of November birds within the group at $p<0.05$ level (Student's '*t*' test).

Table-27: Effect of 24L/0D on Testicular size and Body Mass of photorefractory Red-vented Bulbul, *Pycnonotus cafer stanfordi* following transfer from constant 16L/8D daylength.*

Months after Exposure	16L/8D		24L/0D	
	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*
00	37.24±0.46	0.83±0.04	36.80±0.21	0.83±0.04
01	37.38±0.56	0.83±0.04	36.76±0.20	0.81± 0.05
02	36.34±0.54	0.87±0.05	35.98±0.16	0.83±0.04

*Values are Mean±*SEM*; n=4 in each group.

CHAPTER – IV

Role of Thyroid and Photoperiod in the Regulation of Gonadal and Body Mass Cycles of Red-Vented Bulbul, *Pycnonotus cafer stanfordi* Deignan at Lumami, Zunheboto District, Nagaland State (Lat. 26º13’ N, Long. 94º28’ E).

INRODUCTION

In many avian species, thyroid ablation inhibits annual gonad development cycles (see reviews: Thapliyal, 1969, 1978, 1981; Assenmacher, 1973; Assenmacher and Jallageas, 1978, 1980; Nicholls *et al*, 1988; Thapliyal and Gupta, 1989; Yashimura, 2013). However, in spotted munia, *Lonchura punctulata* (Thapliyal and Pandha, 1967a), lal munia, *Estrilda amandava* (Thapliyal, and Pandha, 1967c), black-headed munia, *Munia malacca mallaca* (Thapliyal and Pandha, 1967b), female weaver bird, *Ploceus philippinus* (Thapliyal and Bageshwar, 1970) and migratory starling, *Sturnus vulgaris* (Goldsmith and Nicholls, 1984b; Nicholls *et al*, 1988), thyroidectomy results precocious development of gonads which remain active for long or post–breeding regression is wholly wanted. Birds like house sparrow, *Passer domesticus* (Lal and Thapliyal, 1982b), common myna, *Acridotheres tristis* (Chaturvedi and Thapliyal, 1979), and red-headed bunting, *Emberiza bruniceps* (Lal, 1988), respond to thyroidectomy differently depending on their sexual stage at the time of surgery. Further, in majority of birds, thyroid ablation inhibits long day induced gonadal enlargement (see reviews, Höhn, 1961; Thapliyal, 1969, 1978, 1981; Assenmacher, 1973; Assenmacher and Jallageas, 1978, 1980; Nicholls *et al*, 1988; Thapliyal and Gupta, 1989; Yashimura, 2013). In sharp contrast, thyroidectomy in weaver bird, *Ploceus philippinus,* promotes follicular growth in birds kept on non-stimulatory short daylengths (Thapliyal and Bageshwar, 1970).

Treatments with moderate/physiological doses of thyroid hormones successfully counter the effects of thyroidectomy on gonads, but higher doses prompt gonadal involution in most of the species studied so far (see reviews, Thapliyal 1981; Nocholls *et al*, 1988; Thapliyal and Gupta, 1989; Yashimura, 2013). Birds like red-headed bunting, *Emberiza bruniceps* (Thapliyal and Lal, 1984), common myna, *Acridotheres tristis* (Chaturvedi and Thapliyal, 1980a,b) and black-headed bunting, *Emberiza melanocephala* (Devi and Lal, 1994), house sparrow, *Passer domesticus* (Miller, 1935; Vaugien, 1954) and lal munia, *Estrilda amandava* (Thapliyal and Gupta, 1984) influence of thyroid hormones on gonads vary during different reproductive states.

Great diversity has been observed amongst species with regard to involvement of thyroid hormones in influencing their post-reproductive termination of gonads. In some bird species, surgical ablation of thyroid glands and/or exogenous treatments of thyroid hormones produce no effects on naturally decreasing gonads. Such examples include birds like red-headed bunting, *Emberiza bruniceps* (Lal, 1988), tree sparrow, *Passer montanus* (Lal and Pathak, 1987), common myna, *Acridotheres tristis* (Chaturvedi and Thapliyal, 1979, 1980a), house sparrow, *Passer domesticus* (Lal and Thapliyal, 1982b) and black-headed bunting, *Emberiza melanocephala* (Devi and Lal, 1992, 1994). On the contrary, gonads of Japanese quail, *Coturnix coturnix japonica* (Follett and Nicholls, 1984; Chaturvedi and Meier, 1989), duck, *Anas platyrynchos* and teal, *Anas crecca* (Assenmacher and Jallageas, 1978, 1980), white crowned sparrow, *Zonotrichia leucophrys gambelii* (Smith, 1978, 1980) and European starling, *Sturnus vulgaris* (Nicholls *et al*, 1988), re-develop following extirpation of thyroid glands and post-nuptial gonadal regression fail to ensue. In American tree sparrows, *Spizella arborea*, thyroid hormones are reported to act centrally in programming the development and dissipation of breeding season (Wilson and Reinert, 2000).

Similar to gonads, body weight regulating mechanisms respond differently to the surgical ablation of thyroid glands. Three types of body weight responses to thyroidectomy have been observed in birds studied so far. Thyroidectomy induces gain in body weight of birds like chestnut-bellied munia, *Munia atricapoilla* (Thapliyal and Garg, 1967), lal munia, *Estrilda amandava* (Thapliyal, 1969), spotted munia, *Lonchura punctulata* (Thapliyal, 1969), weaver bird, *Ploceus philippinus* (Thapliyal and Garg, 1969; Thapliyal and Bageshwar, 1970), European starling, *Sturnus vulgaris* (Weiselthier and Van Tienhoven, 1972) and rain quail, *Coturnix coturnix coromandelica* (Saxena and Saxena, 1975). In sharp contrast, body weight of

Indian starling, *Sturnus roseus* (Shah *et al*, 1977), Indian spotted dove, *Streptopelia chinensis* (Malik and Lal, 1998) and common grey quail, *Coturnix coturnix* (Saxena and Saxena, 1979) decrease following excision of thyroid glands. In red-vented bulbul, *Molpastes cafer* (Lal and Thapliyal, 1982a), common myna, *Acridotheres tristis* (Chaturvedi and Thapliyal, 1979, 1980a), house sparrow, *Passer domesticus* (Lal and Thapliyal, 1982b) and tree sparrow, *Passer montanus* (Lal and Pathak, 1987) thyroidectomy has no effects on body weight of birds. An added complication describes the influence of thyroidectomy on body weight of birds to differ depending upon physiological status of birds at thyroid extirpation. So far, such responses have been noticed only in sub-tropical buntings like *Emberiza bruniceps* and *Emberiza melanocephala*. In these birds, thyroidectomy during progressive and peak phases of the body mass cycle result rapid decline in body weight of birds, but has no effects when surgery is performed during regression and/or quiescent phases (Lal and Thapliyal, 1985a; Lal, 1988; Devi and Lal, 1992, 1994).

Every species seems unique in making use of thyroid hormones to fine tune its ecological needs like migration, molt and reproduction. Current literature though derived largely from studies on Japanese quail, *Coturnix coturnix japonica* state that long daylengths (LD) enhance the expression of type 2 deiodinase (Dio2) gene which up regulates synthesis of thyroid hormone activating enzyme for effective conversion of T_4 to T_3 (Bernal, 2002) within mediobasal hypothalamus (MBH) (Yashimura *et al*, 2003). On the contrary, short days (SD) uphold the expression of type 3 deiodinase (Dio3) gene responsible for promoting conversion of T_4 to physiologically inactive reverse-T_3 (r-T_3) and T_2 (3, 3' diiodotyrosine) (Yasuo *et al*, 2005). Dio2 and Dio3 gene expression in the MBH under LD and SD seem to regulate seasonality in photoperiodic birds and mammals. Intracerebroventricular infusion of T_3 in Japanese quail kept on SD was found to induce testicular recrudescence in a dose dependent manner. This response disappeared when Dio2 gene expression was blocked with iopanoic acid (Yashimura *et al*, 2003). Dio2 and/or Dio3 gene expressions have been observed to vary across seasons in Eurasian tree sparrows (Watanabe *et al*, 2007), jungle fowl (Ono *et al*, 2009), great tits (Perfito *et al*, 2012) and canary (Stevenson and Ball, 2012). Studies involving electron microscopy of median eminence of Japanese quail reveal opposing morphological features in neurons secreting gonadotrophin releasing hormone (GnRH) and the glial endfeet in birds kept on LD and SD (Yamamura *et al*, 2004). In LD birds GnRH neurons were found to converge on basal lamina (Prevot *et al*, 1999) which was seen obstructed by thick glial endfeet in quails kept on SD (Yamamura *et al*, 2006). Normal structural anatomy of GnRH neurons and glial cells could be restored in SD quails by hypothalamic infusion of T_3. Seasonal diversity encountered in structural features of GnRH neurons and the glial endfeet in birds appear to have great analogy with ewes (Jansen *et al*, 2003). A consensus seems to be evolving around T_3 as the key regulator of structural changes in GnRH neurons and glial cells to modulate seasonal output of GnRH in birds and mammals. However, Dio2 gene expression is not correlated with seasonal increase and decrease in testes of European starlings, *Sturnus vulgaris* (Bentley *et al*, 2013). This has prompted necessity to redefine the concept with broader perspectives accommodating scanning of many more species to design unequivocal concept. Fresh insight needs to be placed to resolve possible involvement of thyroid hormones in cross talk between neuroendocrine-gonadal and neuroendocrine-metabolic axes during adaptations to newer habitats and the possible trade-off to elevate survival fitness of species. It was therefore, decided to examine the involvement of light and thyroid hormones in the development and dissipation of gonads and body mass of red-vented bulbul, *Pycnonotus cafer stanfordi* populations in and around Lumami, Zunheboto district.

MATERIALS AND METHODS

Wild caught red-vented bulbuls were acclimatized to laboratory conditions for at least a fortnight prior to their use. A set of four experiments were executed with definite scientific queries.

Experiment–I. This experiment was performed to assess the effect of surgical ablation of thyroid glands on annual gonadal and body mass cycles of red-vented bulbuls, *Pycnonotus cafer standfordi* under natural lighting (NDL) at Lumami, Zunheboto district (26^{o}N). During the 2nd week of January 2019, body mass and gonad size of individual birds were recorded. Two groups of male and 02 groups of female with 06 birds in each group were established. One group of male and one group of female was subjected to surgical removal of thyroid glands. The remainder two groups, one each of male and female birds were sham-thyroidectomized. All the groups were maintained in an indoor aviary which received unrestricted light and air. Birds were maintained under NDL and monthly records on body mass and left testis or largest follicle size *in situ* of individual birds were maintained until October 2019.

Experiment-II. This experiment aimed at evaluation of effects of thyroidectomy at different stages of photoinduced gonadal and body mass cycles of red-vented bulbuls. During 3rd week of January 2020, 20 male and 16 female birds were weighed individually and laparotomized for measuring the left testis or the largest follicle size *in situ*. All these birds were transferred to constant 16L/8D photoschedule. Five males were surgically thyroidectomized on day 0 and 1, 2, 4 months after photostimulation when testes were small and inactive (quiescent phase), developing (progressive phase), developed (peak phase) and declining (regression phase). Similarly, a group of 04 females was subjected to surgical thyroidectomy on day 0 and 1, 2, 3 months after photostimulation by 16L/8D when ovarian follicles were small, developing, developed and regressing. Body mass and left testis or the largest follicle size *in situ* of individual male and female birds were kept regularly at monthly intervals for 5 and 4 consecutive months respectively.

Experiment- III. This experiment was made to assess whether intermittent replacement therapy with L-thyroxine (L-T_4) in thyroidectomised birds would influence in any way, the total length of photoinduced sexual and body mass cycles of red-vented bulbuls. During the second week of March 2020, 03 groups with 05 birds in each were established. Individual body mass and gonad size *in situ* of all the birds were kept and thereafter, 02 groups of male and 02 groups of female birds were subjected to surgical thyroidectomy. The remainder groups, one each of male and female birds were sham-thyroidectomized. All the groups of birds were transferred to light boxes with fixed durations of 16L/8D. Following day, a group of thyroidectomised male and female birds was separately administered with L-thyroxine at 1µg/bird/day in slightly alkaline saline at monthly gaps. One group each of thyroidectomised and sham-operated male and female birds received 0.1 ml of the vehicle only and served as the control. The experiment was continued to cover one photoinduced gonadal cycle before termination in August 2020.

Experiment-IV. This experiment was performed to assess if dissipation of photorefractoriness in thyroidectomised birds occur like sham-operated intact birds? Possible solution to this query was sought by establishing 02 groups of male and 02 groups of female thyroidectomised photorefractory birds under constant 16L/8D daylength. These groups, along with a group of intact males and a group of female birds photorefractory to 16L8D, were individually weighed laparotomized to measure the length and width of left testis or the diameter of largest follicles *in situ*. All the groups were transferred to constant 8L/16D daylength for 10 weeks. After 10 weeks, individual records on gonad size and body mass were made and birds were shifted to constant 16L/8D photoperiodic regimen. One group of male and onother group of female thyroidectomised birds were treated with L-T_4 at 1µg/bird/day in 0.1 ml normal saline. One group each of intact and thyroidectomised males and similar groups of intact and thyroidectomised females were injected with 0.1 ml of the vehicle only. Treatments were continued for 3 consecutive months. Records on individual body mass and gonads size of all the groups were kept regularly at monthly intervals.

Surgical ablation of thyroid glands was performed under the influence of Sodium Pentobarbital (Nembutal, Schering India Ltd., 20mg/kg body mass) following the technique described by Lal (1988). The ventral side of the neck region between head and keel was deplumed. An incision was placed on the ventrolateral sides to expose thyroid glands which are adhering one each to the left and right carotids. The thyroid glands were removed with the help of two fine forceps. In sham-thyroidectomized birds, thyroid glands were located and touched with the forceps. The incision was sutured with fine silk thread and the wound was dusted with Neosporin (Johnson and Johnson) powder to prevent infection. Laparotomy technique followed was as described in Chapter II. At surgery, a small area of the chest was deplumed and completion of thyroidectomy was ensured with the lack of feather regeneration in thyroidectomised birds. In long-run some birds had regenerated feathers which were small, white or brownish in colour and lacked barbules. L-thyroxine (Sigma Chemical Company, St. Louis, USA) was dissolved in 0.5 ml of 0.01N NaOH and then diluted with normal (0.9%) saline to the desired concentration. L-thyroxine (L-T_4) dose was fixed based on our earlier experience that this concentration fully reverses the effects of thyroidectomy on gonads and body mass of red-vented bulbuls (Neelakshi, unpublished). Injections were made on the alternate sides of breast and thigh muscles in 0.1ml saline daily between 8.30-9.0 am only (2.5-3.0 hrs after light was switched on). Temperature difference in the light boxes and the ambient conditions did not exceed 1.5^{oC}. Light intensity at the perch level and husbandry conditions were as described in Chapters II and III.

At the termination of experiments, some birds were sacrificed but only when it became a necessity to do so in support of crucial assumptions or otherwise they were rehabilitated and set free in the wild in good health.

Statistical techniques used for data analysis were based on independent measure of analysis of variance (ANOVA). Student's *'t'* test was employed to compare between groups values (Snedecor, 1961).

RESULTS

Experiment–I. Effects of Thyroidectomy on Sexual and Body Mass Cycles: One way repeated measures ANOVA revealed a significant seasonal variation in the size of gonads of both the sexes of bulbuls; male: $F_{(11,60)}$= 26.42, p<0.0001, and female: $F_{(11,60)}$= 96.32, p<0.0001. Testes of birds began developing during February-March, attained peak in May (120.36±11.38 mm^3) and declined thereafter to reach seasonally low value during October (Table 28, Fig. 24). Testicular size in May and June measured significantly more compared with values obtained in other months (Table 28, Fig. 24). Thyroidectomy performed during January inhibited seasonal increase in testicular size as compared to sham-operated birds (One way independent measure of ANOVA, P<0.0001). However, testicular cycle with very small peak (5.24±0.62 mm^3; F($_{11,60}$)= 3.44, p<0.05) appeared in thyroidectomized birds during the month of June. Mean testicular sizes of thyroidectomized birds from May to July periods were significantly more as compared to mean values in other months. Ovarian follicles developed progressively during February and March, peaked in June followed by regression reaching seasonal low value in October (Table, 29, Fig. 25). Thyroidectomy suppressed seasonal follicular growth. However, a follicular cycle with very low amplitude (0.66±0.15mm; F($_{11,60}$)=2.44,p<0.05) in June appeared (Table 29, Fig. 25). Within group follicular size of thyroidectomised birds between May-July was significantly higher as compared to values observed in other months (Table 29, Fig. 25).

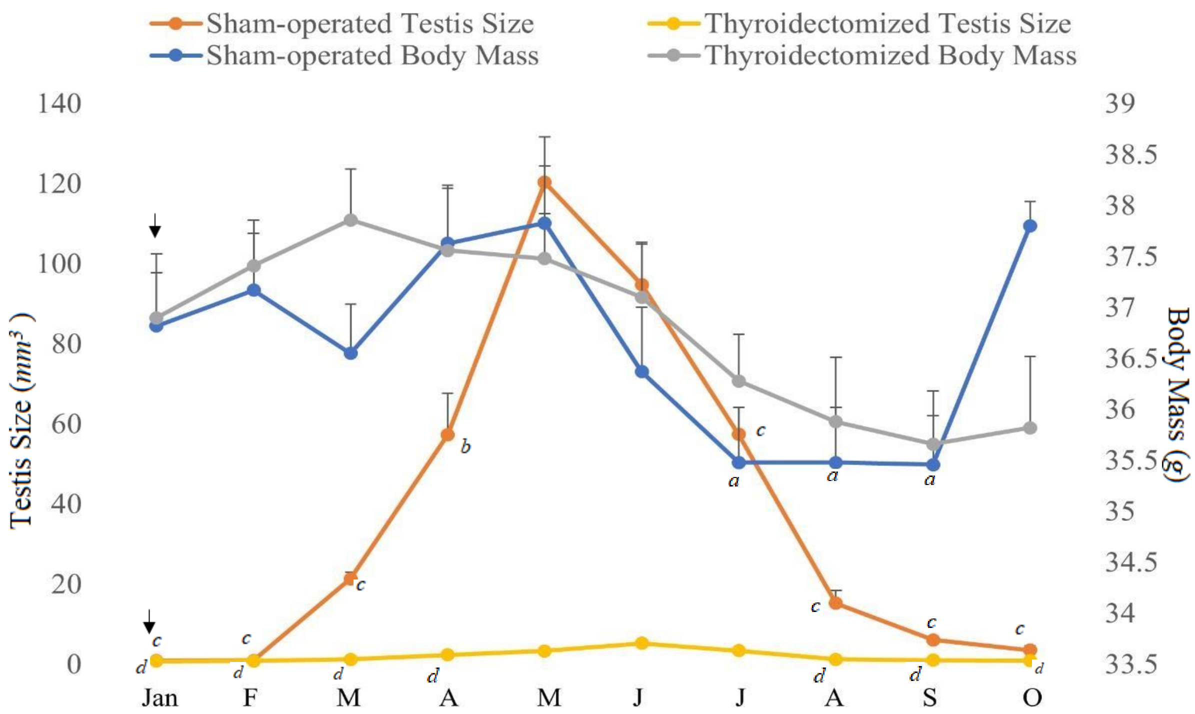

Fig24: Seasonal changes in Testicular Size and Body Mass of Sham-operated and Thyroidectomized Red-vented bulbul, *Pycnonotus cafer stanfordi*, Values are Mean±*SE*; n=6 in each group. Body Mass: Sham-operated, F$_{(11,60)}$=4.64, p<0.01; Testicular Volume: F$_{(11,60)}$ = 26.42, p<0.0001; Body Mass: Thyroidectomized, F$_{(11,60)}$=2.64, non-significant; Testis Size F$_{(11,60)}$= 3.44 p<0.05 (ANOVA). *a,b,c* differ from value of May month at p<0.05, <0.01 and <0.001 respectively . *d* differs from the value of Thx in June at p<0.05 (Student's *'t'* test).

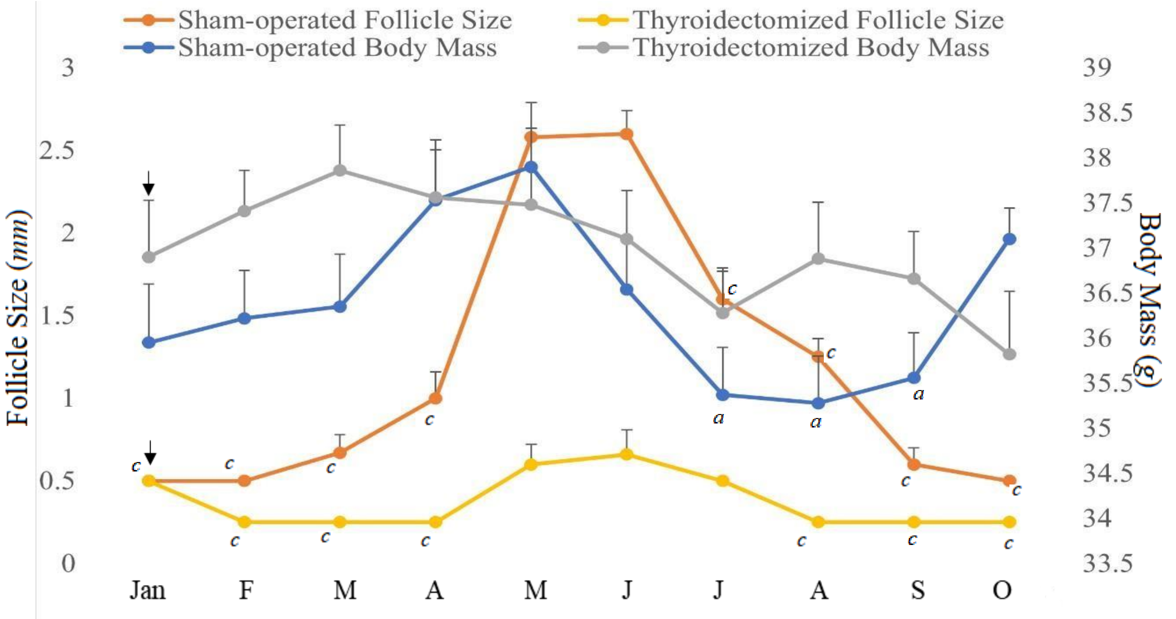

Fig25: Effect of Thyroidectomy (Thx) and intermittent treatments of L-Thyroxine (L-T$_4$) on Testicular Size and Body Mass of Red-vented bulbul, *Pycnonotus cafer stanfordi*. Seasonal changes in Follicular Size and Body Mass of Sham-operated and Thyroidectomized Red-vented bulbul, *Pycnonotus cafer stanfordi* .Values are Mean±*SE*; n=6 in each group. Body Mass, sham-operated: F$_{(11,60)}$=5.64, p<0.001; Follicle Size: F$_{(11,60)}$ = 96.32, p<0.0001; Body Mass, Thyroidectomized: F $_{(11,60)}$=1.54, non-significant; Follicle Size F$_{(11,60)}$= 2.44, p<0.05 (ANOVA). *a,c* differ from within group values of May at p<0.05, and <0.001 respectively (Student's *'t'* test).

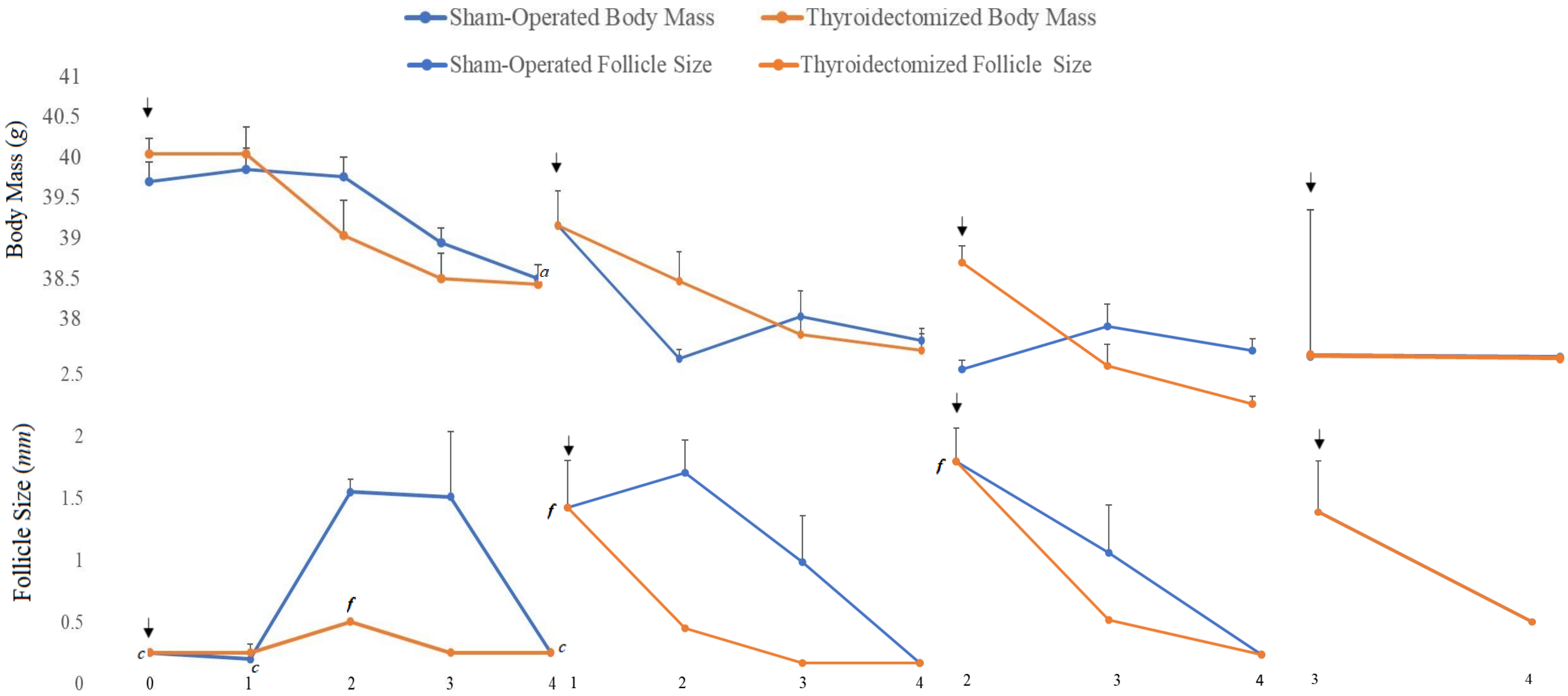

Fig.27: Effect of Thyroidectomy (Thx) at different stages of a Photoinduced Follicular and Body Mass cycle of Red-vented bulbul, *Pycnonotus cafer stanfordi*. Values are Mean±SE. ↓indicates point of Thx. *a* differs from body mass of sham-operated group after 2m at $p<0.05$ level. *c* differs from value of testis size of intact birds after 2m at $p<0.001$. *f* differs from testis size of Thx on day 0 at $p<0.001$ level (Student's '*t*'test).

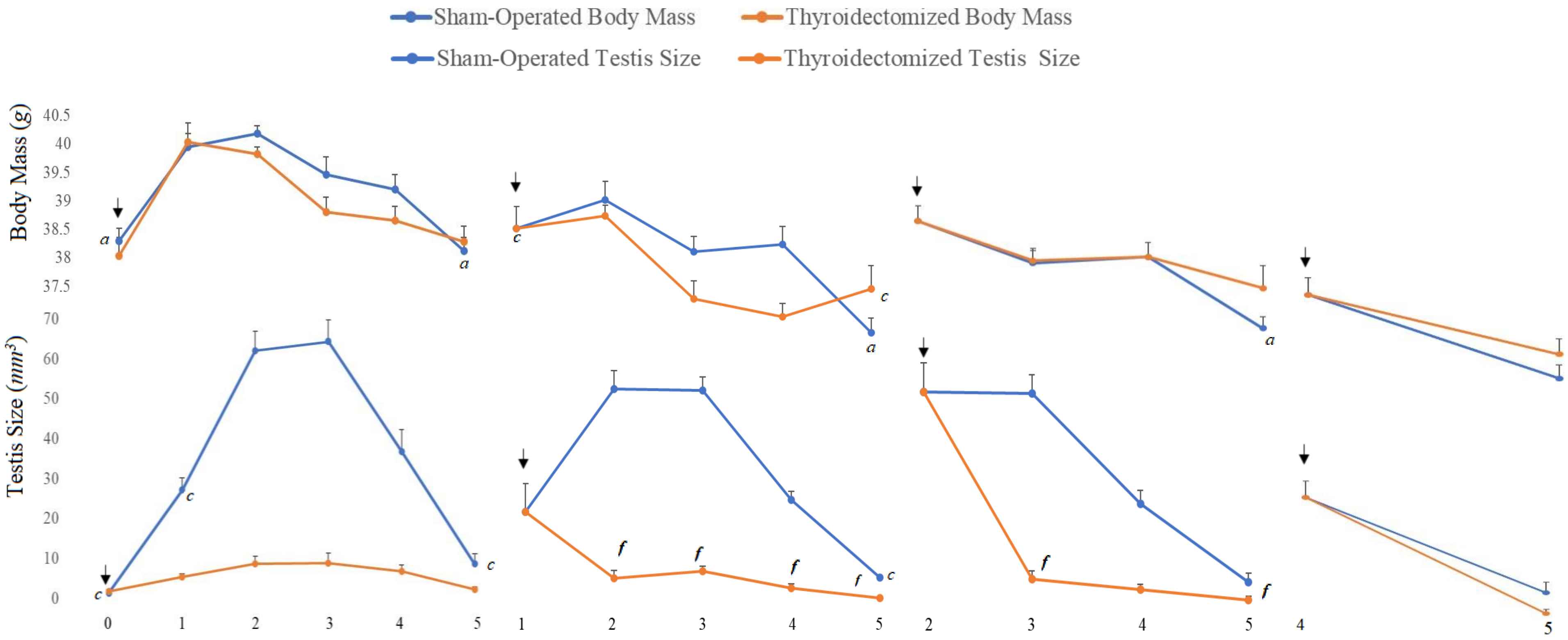

Fig.26: Effect of Thyroidectomy (Thx) at different stages of a Photoinduced Testicular and Body Mass cycle of Red-vented bulbul, *Pycnonotus cafer stanfordi*. Values are Mean±*SE*. ↓indicates point of Thx. *a* differs from body mass of intact birds after 2m at $p<0.05$. *c* differs from value of testis size of intact birds after 2m at $p<0.001$. *f* differs from testis size of Thx birds on day 0 at $p>0.001$.

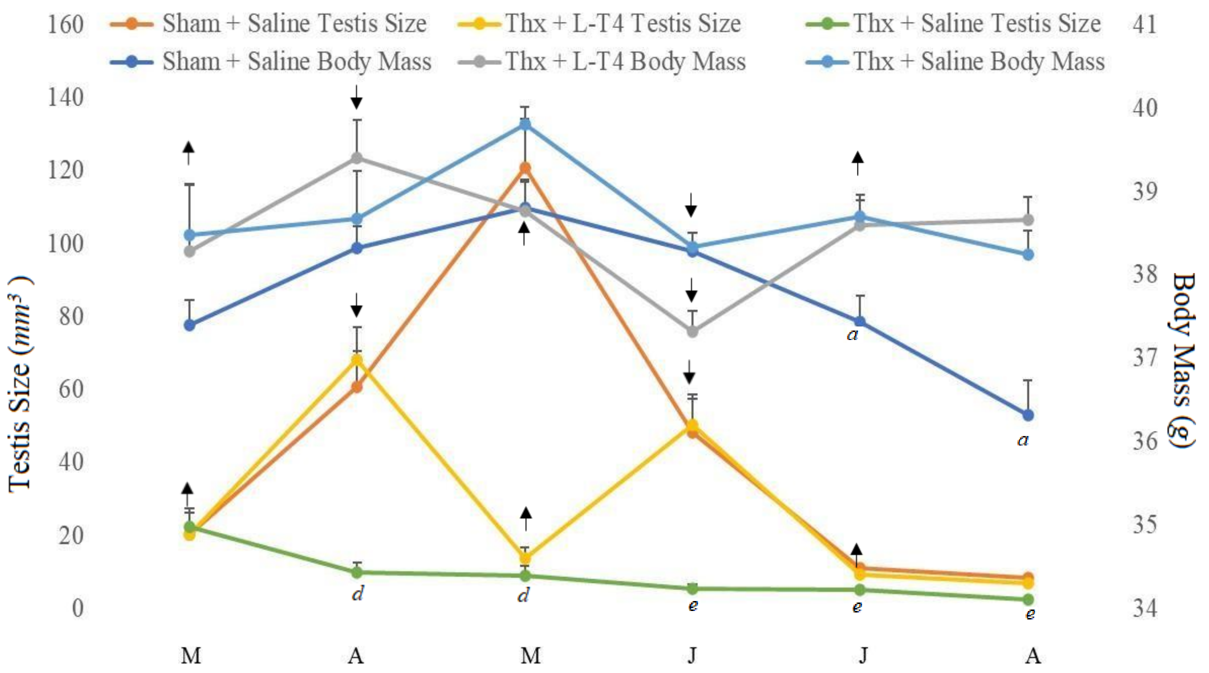

Fig28: Effect of Thyroidectomy (Thx) and intermittent treatments of L-Thyroxine (L-T_4) on Testicular Size and BodyMass of Red-vented bulbul, *Pycnonotus cafer stanfordi.* Values are expressed as Mean±*SE*; ↑ shows L-T4 on (1µg/bird/day) and ↓ shows L-T4 off; n=5 in each group a differs from the value of May month at p<0.05 level. *d,e* differ from the value of Thx birds in March at p<0.05 and <0.01 respectively (Student's *'t'* test).

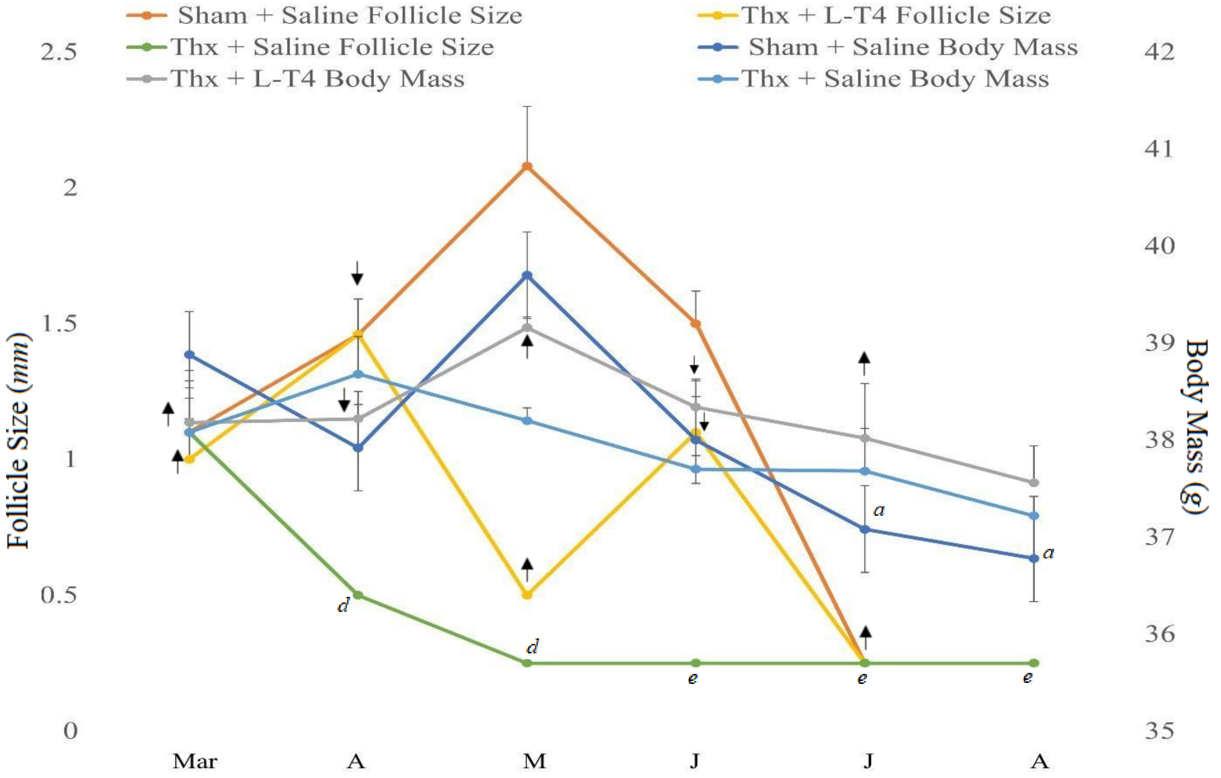

Fig.29: Effect of Thyroidectomy (Thx) and interrupted treatment of L-Thyroxine (L-T_4) on and Follicle size and Body Mass of Red-vented bulbul, *Pycnonotus cafer stanfordi.* Values are represented as Mean±*SE*; St, stimulated; n=5 in each group. ↑shows L-T4 on (1µg/bird/day), ↓shows L-T4 off. *a* differs from the value of May month at p<0.05 level. *d, e* differs from within group value of Thx birds at p<0.05 and 0.01 level respectively (Student's *'t'* test).

One way repeated measures ANOVA revealed significant seasonal variation in the body mass of both the sexes of bulbuls; male: F($_{11,60}$)=4.64, p<0.01, and female: F$_{(11,60)}$= 5.64, p<0.001). Body mass of sham-operated male birds was recorded maximum during May (37.83±0.56g) which was significantly more as compared to within group values between June-August (Table 28, Fig.24). Seasonal changes in body mass was abolished by surgical ablation of thyroid glands in both the sexes; male: F($_{11,60}$)=2.64, non significant; female: F ($_{11,60}$)=1.54, non significant. In general, mean body mass of thyroidectomised birds was more compared with sham-operated groups (Tables 28,29; Figs.24 &25).

Experiment- II. Effect of Thyroidectomy at different stages of a photoinduced Gonadal and Body Mass Cycle: Gonads of sham-operated birds developed maximally within 02 months of exposure to constant 16L/8D, and thereafter declined to reach low size within 4-5 months of light treatment. Thyroidectomy performed on day 0 severely suppressed the amplitude of gonadal growth in both the sexes of birds. However, temporal pattern of partially developed testes and ovarian follicles in thyroidectomised groups were similar to the sham-operated birds (Tables 30- 31, Figs.26, 27). Thyroidectomy performed on birds with developing and developed gonads after 1 and 2 months of exposures to 16L/8D resulted in instant gonadal regression and gonads declined to reach to the level of maximally developed gonads of birds thyroidectomised on day 0 (Tables 30-31, Figs. 26-27). However, thyroidectomy performed on birds with regressing gonads did not have any effect as the gonads in sham-operated and thyroidectomised birds regressed at similar pace (Tables 30-31, Figs. 26 - 27).

Body mass of sham-operated birds was maximum at 2 months after exposure to constant 16L/8D and measured significantly (p<0.05) more as compared to values in male birds on day 0 and 5 months after exposure to long daylength (Tables 30-31, Figs 26-27). Thyroidectomy performed at different stages of a photoinduced body mass cycle failed in influencing body mass of both the sexes of red-vented bulbuls (Tables 30-31, Figs.26- 27).

Experiment-III: Effect of thyroidectomy and intermittent replacement with L-T_4 on development of Photorefractoriness: Gonads of sham-operated birds exposed to constant 16L/8D daylength during March grew rapidly to reach a peak in May which was followed by regression to reach a low value in August (Tables 32-33, Figs. 28-29). Thyroidectomy suppressed developing gonads of birds transferred to constant 16L/8D during the month of

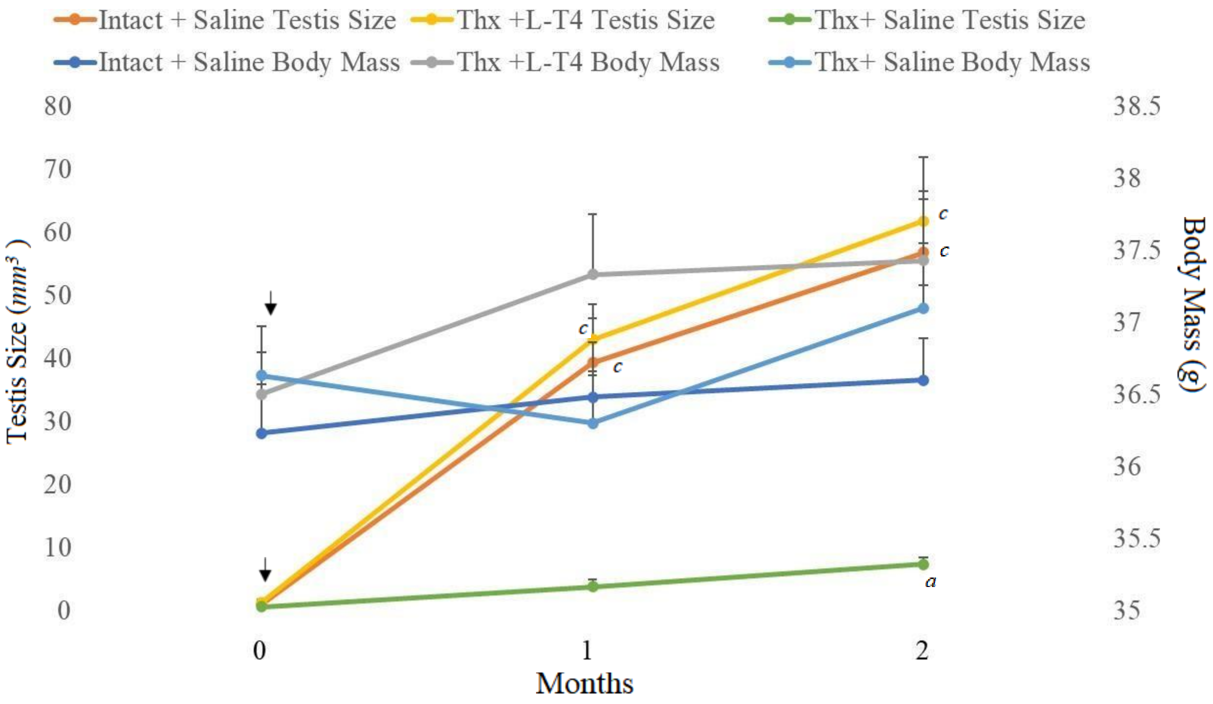

Fig.30: Effect of Daily Treatments of L-T_4 on Testicular Size and Body Mass of Thx Photorefractory Red-vented bulbul, *Pycnonotus cafer stanfordi*. Values are Mean±*SE*; n=4 in each group. L-T4= L-thyroxine (1µg/bird/day); Thx, Thyroidectomized *a,c* differ from value of day 0 at p<0.05and p<0.001 respectively (Student's *'t'* test).

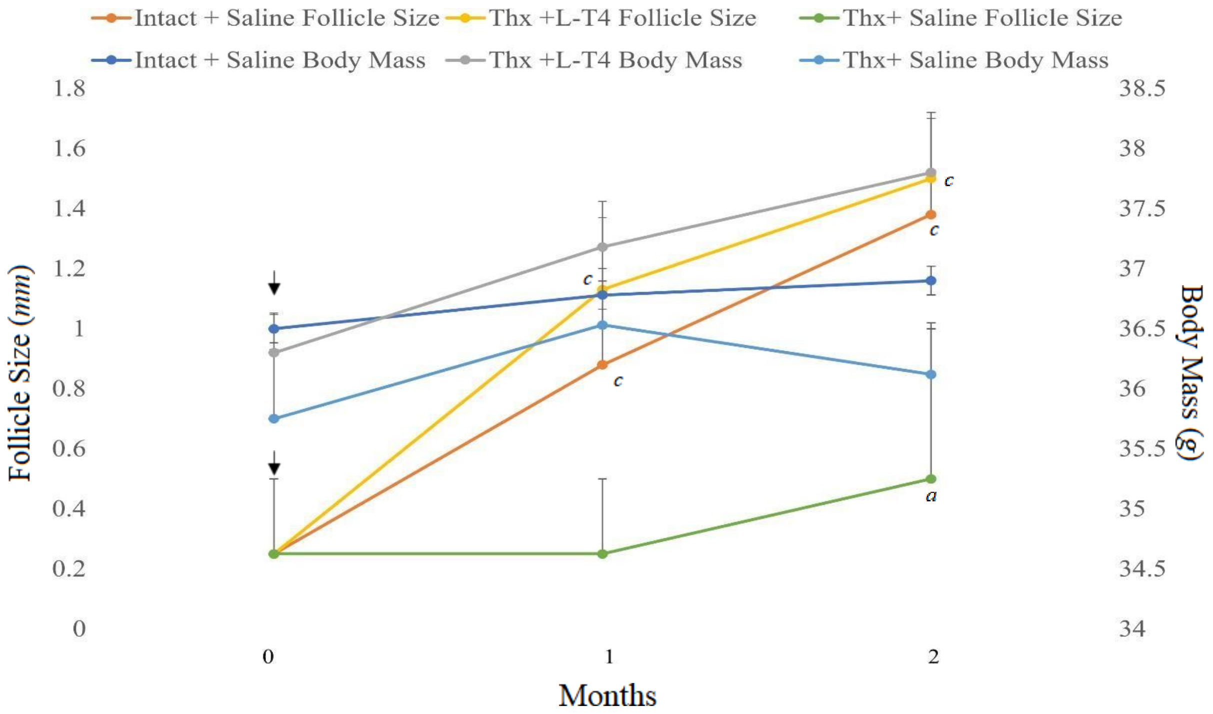

Fig.31: Effect of Daily Treatments of L-T_4 on Follicular Size and Body Mass of Thx Photorefractory Red-vented bulbul, *Pycnonotus cafer stanfordi*.Values are mean±*SE*; n=4 in each group; L-T4= L-thyroxine (1µg/bird/day); Thx, Thyroidectomized *a,c* differ from value of day 0 at p<0.05andp <0.001 respectively (Student's *'t'* test).

March. Further, intermittent treatments of L-T_4 at 1 µg/bird in thyroidectomised birds led to recovery of gonads to the level of sham-operated group in the contemporary month and withdrawal of injections resulted in rapid decrease of gonads to the extent of thyroidectomised group receiving saline. L-T_4 treatments in thyroidectomised birds during July-August (4 months after exposure to 16L/8D) failed in preventing gonadal regression (Tables 32-33, Figs. 28=29). Mean body mass of sham-operated birds measured maximum at 02 months of exposure to constant 16L/8D photoperiod (during May) which was significantly more as compared to mean body mass of birds after 4-5 months of exposure to 16L/8D (during July and August). Thyroidectomy and/or intermittent treatments with L-T_4 at 1 µg/bird/day did not influence body mass of thyroidectomized birds (Tables 32-33, Figs. 28-29).

Experiment-IV: Short day induced recovery of photosensitivity in intact and thyroidectomised birds: Testes of photorefractory intact birds treated for 10 weeks with constant 8L/16 daylength developed significantly following transfer to constant 16L/8D photo-schedule. Testes of photorefractory thyroidectomised birds pre-exposed to 8L/16 for 10 weeks and injected with saline developed significantly over their initial values only after two months of exposure to constant 16L/8D daylength. L-T_4 treatments in thyroidectomised birds at daily dose of 1 µg/bird led to the development of testes on par with intact birds (Tables 34-35, Figs.30-31). Ovarian follicles of photorefractory intact birds pre-treated with 8L/16D for 10 weeks developed significantly over their initial values following transfer to 16L/8D photoperiod. Significant follicular increase over their initial value in thyroidectomised birds pre-exposed to 8L/16D for 10 weeks was witnessed only after 2 months following exposure to 16L/8D. L-T_4 treatments at daily dose of 1 µg/bird led to the development of ovarian follicles on par with intact birds treated with saline. No significant within or between group differences in the mean body mass of birds was observed at the end of 10 weeks under 8L/16D and following transfer to constant 16L/8D daylength (Tables 34-35, Figs 30-31).

DISCUSSION

Gonads : Present results show that gonads of both the sexes of red-vented bulbuls, *Pycnonotus cafer stanfordi* developed progressively from March to May, peaked in May/June and thereafter declined to small size during autumn months. Extirpation of thyroid glands suppressed seasonal development of gonads in birds kept on NDL. Further, although gonads of thyroidectomized birds had developed partially only, temporal patterns of their increase and decrease were as in intact group. This response of gonads in red-vented bulbuls to thyroidectomy is in conformity with the observation of Lal and Thapliyal (1982a) on red-vented bulbul populations studied in the northern part of India (26°N). Thyroidectomy has also been reported to inhibit or retard development of gonads in many other avian species which include birds like male baya, *Ploceus phillipinus* (Thapliyal and Garg, 1969), Eurasian tree sparrows, *Passer montanus,* common myna, *Acridotheres tristis,* Indian spotted dove, *Streptopelia chinensis,* house sparrows, *Passer domesticus* and red-headed bunting, *Emberiza bruniceps,* black-headed buntings, *Emberiza melanocephala* and American tree sparrows, *Spizella arborea* (Chaturvedi and Thapliyal, 1979, 1980; Lal and Thapliyal, 1982b; Nicholls *et al*, 1988; Lal and Pathak, 1987; Lal, 1988; Devi and Lal, 1992; Reinert and Wilson, 1996a,b, 1997a,b; Wilson and Reinert, 1999, 2000; Malik and Lal, 1998). In all these species, thyroidectomy suppresses enlargement of gonads without distorting basic features of seasonality in development and regression. This denotes that these avian species need functional thyroid glands for optimal secretion of GnRH. In sharp contrast, gonads of some avian species develop following removal of thyroid glands and in them, post-breeding regression is either delayed or gonads remain active for indefinite periods. This group includes birds like chestnut–bellied munia, *Munia atricapilla* (Thapliyal and Garg, 1967), lal munia, *Estrilda amandava* (Thapliyal and Pandha, 1967c), spotted munia, *Lonchura punctulata* (Thapliyal, 1969), female baya, *Ploceus philippinus* (Thapliyal and Bageshwar, 1970) and migratory starlings, *Sturnus vulgaris* (Wieselthier and Van Tienhoven, 1972; Goldsmith and Nicholls, 1992; Dawson, 1993). An added complication adding to conceptual intricacy focuses on reports that in some avian species response of neuroendocrine-gonadal axis to thyroidectomy differs depending upon the season and/or the sexual stage at surgery (Chaturvedi and Thapliyal, 1980a,b; Lal and Thapliyal, 1982a,b; Lal and Pathak, 1987; Lal, 1988; Devi and Lal, 1992).

The observation that thyroidectomy performed during the quiescent phase had suppressed photoinduced enlargement of gonads and rapid gonadal decline ensued following extirpation of thyroid glands in progressive and peak phase gonads on constant 16L/8D daylength confronts with reports in common myna, *Acridotheres tristis* in which daylength in excess of 15L/9D successfully reverses inhibitory influence of thyroidectomy on developing and developed gonads of birds under NDL (Chaturvedi and Thapliyal, 1980a, 1983). Further, thyroidectomy performed in birds with maximally developed gonads under NDL extends their active phase for at least a month (Chaturvedi and Thapliyal, 1983). Present response of gonads of red-vented bulbuls to thyroidectomy finds close similarity with observations presented in migratory red-headed and black-headed buntings in which thyroidectomy suppressed developing and developed testes of birds maintained at constant 15L/9D (Lal and Thapliyal, 1985; Devi and Lal, 1992, 1994). Thus, it is obvious that development of gonads and maturation of the reproductive system in red-vented bulbuls are thyroid hormone dependent physiological events. Lack of an effect of thyroidectomy on regressing gonads of birds under 16L/8D photoperiod might thus represent thyroid hormone independent transition of birds from breeding to non-breeding phase. The fact that intermittent treatments of L-T_4 successfully countered inhibitory influence of thyroidectomy on developing and developed gonads, but failed in influencing post-nuptial decline of gonads of birds kept on 16L/8D lends support to this suggestion. No response of gonads to thyroidectomy and/or L-T_4 treatments during post-nuptial regression of testes has also been reported in red-headed and black-headed buntings (Lal and Thapliyal, 1985; Devi and Lal, 1992, 1994). However, present observations in red-vented bulbuls contradict findings in European starlings, *Sturnus vulgaris* (Nicholls *et al*, 1988), Japanese quail, *Coturnix coturnix japonica* (Chaturvedi and Meier, 1989), duck, *Anas platyrynchos*, and teal, *Anas crecca* (Assenmacher and Jallageas, 1980) in which thyroidectomy has been reported to prevent transition of birds from breeding to non-breeding state.

During transit from breeding to non-breeding phase, neuroendocrine-gonadal axis of red-vented bulbuls becomes insensitive to photostimulation (see Chapter-III). This response of bulbuls seemingly represents basic feature of birds and mammals which schedule their reproductive timings during spring and/or summer months and breeding ends abruptly due to development of photorefractoriness (Nicholls *et al*, 1988; Thapliyal and Gupta, 1989; Dawson, 2015). Further, cessation of response of gonads to thyroidectomy and/or L-T_4 treatments during regression phase shows that birds were insensitive to thyroid hormones as well. It is difficult to decide whether thyrorefractoriness is a cause or a consequence of development of photorefractoriness? Viewing at recent advances in molecular approaches to light responses of birds and mammals, it may be opined that the former overweighs latter possibility. In Japanese quail, *Coturnix coturnix japonica,* intracerebroventricular (*icv*) infusion of T_3 in birds kept on short days (SD) rendered them photoperiodically blind as testicular recrudescence ensued in a dose dependent manner and the response could be countered by application of ipanoic acid (Yashimura *et al*, 2003). Further, long days (LD) induce synthesis of thyroid hormone receptor subunits α, β, ϰ along with enhanced expression of Dio2 gene which up regulate synthesis of type-II deiodinase enzyme to activate conversion of T_4 (3,5,3'5' tetraiodothyronine) to T_3 (3,5,3' triiodothyronine) (Bernal, 2002) within MBH of birds and mammals (Yashimura *et al*, 2003). In sharp contrast, short daylengths (SD) uphold the expression of Dio3 gene resulting expression of type-III deiodinase to push cascade towards active conversion of T_4 to r-T_3 (reverse T_3) and its subsequent degradation to T_2 (diiodotyrosine) (Yasuo *et al*, 2005). Different responses of Dio2 and Dio3 gene expressions in MBH under LD and SD appear to regulate seasonality in birds nesting during spring and summer months. The fact that changes in Dio2 and/or Dio3 gene expressions associated with the development and regression of gonads supports this view. This concept has been upheld in some species like tree sparrows, *Passer montanus,* great tits, *Parus major,* fowl, *Gallus gallus* and canary, *Serinus canaria* (Watanabe *et al*, 2007; Ono *et al,* 2009; Perfito *et al*, 2012; Stevenson and Ball, 2012; Nabi *et al*, 2020).

Further, studies based on electron microscopy of MBH in Japanese quail have revealed striking differences in structural components of GnRH neurons at the axonal ends. Normal contacts between GnRH neurons and pericapillary space in LD birds was blocked by glial enseathing under SD (Prevot *et al*, 1999) which could be reversed by *icv* infusion of T_3 in birds kept on SD (Yashimura *et al*, 2003). A disconnect between GnRH neurones and pericapillary space or basal lamina owing to enseathing of glial endfeet has also been shown in photorefractory birds (Yamamura *et al*, 2006; Dawson, 2015). Seasonal plasticity in morphological features of GnRH neurons and the glial endfeet have also been observed in the median eminence of ewes (Jansen *et al*, 2003). Striking differences in structural details of glial endfeet in MBH neurons linked with up regulation of Dio2 gene expression and T_3 biosynthesis coinciding development and maturation of gonads followed by elevated expression of Dio3 gene and active deiodination of T_4 to r-T_3 and T_2 (Yasuo *et al*, 2005) though demonstrated most convincingly only in Japanese quail may be tenable in other photoperiodic species with exception of species like European starlings, *Sturnus vulgaris* in which Dio2 gene expression finds no correlation with seasonal testicular recrudescence and/or regression (Bentley *et al*, 2013).

The observation that gonads of intact and thyroidectomised photorefractory birds pre-exposed to 8L/16D for 10 weeks developed significantly following transfer to constant 16L/8D shows that birds had fully recovered from photorefractoriness. In many other avian species 8-10 weeks of exposures to non-stimulatory daylengths have been shown to dissipate photorefractoriness (Dixit and Singh, 2011, 2012; Nicholls *et al*, 1988; Thapliyal and Gupta, 1989; Dawson and Sharp, 2007; Dawson, 2008, 2015). Significant enlargement of gonads in intact and thyroidectomised birds under 16L/8D shows that in red-vented bulbuls, thyroid hormones may not necessarily be involved in transition of birds from photorefractory to photosensitive state. However, presence of thyroid hormones is necessary for full development and maturation of gonads. The fact that L-T_4 treatments resulted full development of gonads supports this view. This however, remains to be established how far synchronized hierarchy in the expressions of Dio2 and Dio3 genes and/or seasonal plasticity of neuronal coordinates tame the functional integrity of photoperiodic response system of red-vented bulbuls?.

Body Mass

Parallel changes in body mass and gonad size of intact birds under NDL and constant 16L/8D daylengths show that body weight and gonadal increase and decrease are regulated by common physical and/or physiological mechanisms. The observation that thyroidectomy permitted only partial gonadal development withought appreciable effects on body mass of birds exposed to natural lighting (NDL) and 16L/8D and these effects could be reversed by exogenous treatments of L-T_4 denotes that in red-vented bulbuls seasonal changes in gonads and body mass are coordinated by thyroid hormones. These results corroborate finding of Lal and Thapliyal (1982a) on body mass of red-vented bulbuls. Present observations fall in line with reports in house sparrow, *Passer domesticus* (Lal and Thapliyal, 1982b), tree sparrow, *Passer montanus* (Lal and Pathak, 1987) and common myna, *Acridotheres tristis* (Chaturvedi and Thapliyal, 1980a) in which thyroidectomy inhibits seasonal development of gonads without having appreciable effects on body mass of birds. In male weaver bird *Ploceus philippinus* surgical ablation of thyroid glands inhibits gonadal growth but increases body mass of birds (Thapliyal and Garg, 1969). In many avian species thyroidectomy influences gonads and body mass in similar ways, for example, in European starling, *Sturnus vulgaris* (Weiselthier and Van Tienhoven, 1972), rain quail, *Coturnix coturnix coromandelica* (Saxena and Saxena, 1975), chestnut-bellied munia, *Munia atricapoilla* (Thapliyal and Garg, 1967; Thapliyal, 1978), lal munia, *Estrilda amandava* (Thapliyal, 1969), spotted munia *Lonchura punctulata* (Thapliyal, 1969), and Indian weaver bird, *Ploceus philippinus* (Thapliyal and Bageshwar, 1970), thyroidectomy increases gonads and body mass both. However, in common grey quail, *Coturnix coturnix* (Saxena and Saxena, 1979), Indian starling, *Sturnus roseus* (Shah *et al*, 1977) and spotted dove, *Streptopelia chinensis* (Malik and Lal, 1998) gonads and body mass of birds decrease together following extirpation of thyroid glands. In some species, influence of thyroidectomy on body weight of birds differs with physiological states at the time of excision of thyroid glands. In migratory red-headed and black-headed buntings surgical removal of thyroid glands during progressive and peak phases of reproductive/body weight cycle results rapid decline in gonads and body mass of birds but has no effects when performed during regression and/or regressed phases (Lal and Thapliyal, 1985a; Lal, 1988; Devi and Lal, 1992, 1994).

Post nuptial decline in body mass seen in intact birds was not apparent in thyroidectomized groups. Further, the fact that post-breeding decline in body mass of birds occurs due to growing insensitivity of metabolic mechanisms to light (also see chapter-III), it may be opined that thyroidectomy might have delayed the onset of metabolic refractoriness. This however, seems unlikely since body mass of thyroidectomized birds also declined although at relatively slow pace as compared to intact birds and L-T_4 administered in these birds did not influence body mass. It may therefore, be opined that slow decline in body mass of thyroidectomized birds might be due to decreased metabolic rate and mobilization of energy reserves at relatively slow pace. Absence of feather renewal in thyroidectomized birds may further add to slow mobilization of energy reserves. There is ample evidence to show diverse functional role of thyroidal hormones in intermediary metabolism of carbohydrates, lipids and proteins in birds (Singh *et al*, 1992,1993, Pucci *et al*, 2000; Lin *et al*, 2003; Kim, 2009; Liu and Brent, 2009; Fliers *et al*, 2010; Welckr *et al*, 2013; McAninch and Bianco, 2014; Sinha *et al*, 2014; Nabi *et al*, 2020). Owing to differential expression of Dio2 and Dio3 genes in the regulation of photoinduced gonadal development and progression towards onset of photorefractoriness in birds coupled with dynamic alterations in morphological features of GnRH nerves in the median eminence, it may not be unreasonable to suggest that similar mechanism might operate during the development and dissipation of photorefractory states – gonadal and metabolic- in red-vented bulbuls. Thus amidst apparent differences in thyroid-gonad and thyroid-body mass relationships in birds, the common narrative entails a crosstalk between neuroendocrine-gonadal and neuroendocrine-thyroidal axes. This linkage inherits credential in bewildering resource mobilization to cope with energy requirements during the course of maturation of the gonads, pair-bonding, courtship, mating, egg laying, incubation and rearing of young ones and thus maximizing the survival fitness of species under diverse ecological habitats.

Table-25: Effect of Simulated 16L/8D and 20L/4D Photoperiods on Testicular size and Body Mass of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan.*

Months 2018	16L/8D		20L/4D		NDL	
	Body Mass	Testicular Size	Body Mass	Testicular Size	Body Mass	Testicular Size
	(g±SE)	*(mm^3±SE)*	*(g±SE)*	*(mm^3±SE)*	*(g±SE)*	*(mm^3±SE)*
October	37.24±0.46	0.83±0.4	36.80±0.21	0.83±0.04	37.62±0.40	0.79±0.00
November	37.38±0.56	2.60±0.67^a	36.76±0.20	3.61±1.13^a	38.24±0.47	0.79±0.00
December	36.34±0.54	1.87±0.35^a	35.98±0.16	1.39±0.40^a	38.42±0.39	0.79±0.00
January	35.32±0.38^a	0.79±0.79	35.32±0.10^a	0.79±0.00	38.02±0.53	0.83±0.04

*Values are Mean±SE; n=5 in each group.

^{a}differs from the value of NDL birds in corresponding months at $p<0.05$ level (Student's '*t*' test).

Table-26 : Effect of Simulated Long days on Testicular size and Body Mass of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan.*

Months *2018*	16L/8D		20L/4D		NDL	
	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*
November	38.10±0.45	0.87±0.05	37.46±0.62	0.83±0.04	37.98±0.45	0.79±0.00
December	38.00±0.44	16.34±2.62^{c}	37.20±0.48	17.20±2.91^{c}	38.28±0.27	0.83±0.04
January	36.00±0.38^{d}	10.72±3.96^{a}	35.20±0.28^{d}	14.34±3.40^{a}	37.68±0.71	0.87±0.05
February	35.22±0.30^{d}	3.28±0.40	34.56±0.19^{d}	5.48±0.12	37.48±0.44	4.36±1.12^{e}

*Values are Mean±*SEM*; n=5 in each group.

[a,c] differ from the value of NDL birds in corresponding months at $p<0.05$ and $p<0.001$ level respectively (Student's '*t*' test).
[e] differs from within group value of testes of NDL birds in February at $p<0.05$ level (Student's '*t*' test).
[d] differs from the mean body mass of November birds within the group at $p<0.05$ level (Student's '*t*' test).

Table-27: Effect of 24L/0D on Testicular size and Body Mass of photorefractory Red-vented Bulbul, *Pycnonotus cafer stanfordi* following transfer from constant 16L/8D daylength.*

Months after Exposure	16L/8D		24L/0D	
	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*	Body Mass *(g±SE)*	Testicular Size *(mm^3±SE)*
00	37.24±0.46	0.83±0.04	36.80±0.21	0.83±0.04
01	37.38±0.56	0.83±0.04	36.76±0.20	0.81± 0.05
02	36.34±0.54	0.87±0.05	35.98±0.16	0.83±0.04

*Values are Mean±*SEM*; n=4 in each group.

Table-28: Seasonal changes in Testicular size and Body Mass of Sham-operated and Thyroidectomized Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan maintained under natural lighting conditions at Lumami (26^{o}N).*

2019	**Sham-operated**		**Thyroidectomized**	
Months	Body Mass *(g±SE)*	Testis Size *(mm^{3}±SE)*	Body Mass *(g±SE)*	Testis Size *(mm^{3}±SE)*
January	36.82±0.52	0.90±0.08^{c}	36.90±0.63	0.79±0.05^{d}
February	37.17±0.56	0.97±0.05^{c}	37.41±0.45	0.86±0.51^{d}
March	36.55±0.48	21.33±1.69^{c}	37.86±0.50	1.23±0.11^{d}
April	37.63±0.54	57.39±10.35^{b}	37.56±0.64	2.39±0.36^{d}
May	37.83±0.56	120.36±11.38	37.48±0.44	3.33±0.28
June	36.37±0.63	94.84±10.12	37.10±0.54	5.24±0.62
July	35.48±0.54^{a}	57.40± 6.80^{c}	36.28±0.46	3.43± 0.30
August	35.48±0.54^{a}	15.22±3.15^{c}	35.88±0.63	1.22±0.25^{d}
September	35.46±0.48^{a}	6.08±0.20^{c}	35.66±0.52	1.08±0.20^{d}
October	37.80±0.24	3.54±1.04^{c}	35.82±0.70	0.94±0.06^{d}

*****Values are Mean±*SEM*; n=6 in each group. Body Mass: Sham-operated, F($_{11,60}$)=4.64, p<0.01; Testicular Volume: F($_{11,60}$) = 26.42, p<0.0001; Body Mass: Thyroidectomized, F($_{11,60}$)=2.64, non significant; Testis Size F($_{11,60}$)= 3.44, p<0.05 (based on one way Independent Measure of ANOVA).

^{a,b,c} differ from value of May month at p<0.05, <0.01 and <0.001 respectively (Student's '*t*' test).

^{d}differs from the value of June month Thx group at p<0.05 (Student's '*t*' test).

Table-29: Seasonal changes in Follicular Size and Body Mass of Sham-operated and Thyroidectomized Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan maintained under natural lighting at Lumami (26^{o}N).*

2019	**Sham-operated**		**Thyroidectomized**	
Months	Body Mass *(g±SE)*	Follicle Size *(mm±SE)*	Body Mass *(g±SE)*	Follicle Size *(mm±SE)*
January	35.95±0.65	0.50±0.00^{c}	36.90±0.63	0.50±0.00
February	36.22±0.53	0.50±0.00^{c}	37.41±0.45	0.25±0.00^{c}
March	36.35±0.58	0.67±0.11^{c}	37.86±0.50	0.25±0.00^{c}
April	37.53±0.56	1.0±0.16^{c}	37.56±0.64	0.25±0.00^{c}
May	37.90±0.43	2.58±0.21	37.48±0.44	0.60±0.12
June	36.54±0.55	2.60±0.14	37.10±0.54	0.66±0.15
July	35.37±0.53^{a}	1.60±0.19^{c}	36.28±0.46	0.50±0.00
August	35.28±0.52^{a}	1.25±0.11^{c}	36.88±0.63	0.25±0.00^{c}
September	35.56±0.50^{a}	0.60±0.10^{c}	36.66±0.52	0.25±0.00^{c}
October	37.10±0.34	0.50±0.00^{c}	35.82±0.70	0.25±0.00^{c}

*****Values are Mean±*SEM*; n=6 in each group. Body Mass, sham-operated: F($_{11,60}$)=5.64, p<0.001; Follicle Size: F($_{11,60}$) = 96.32, p<0.0001; Body Mass, Thyroidectomized: F ($_{11,60}$)=1.54, non significant; Follicle Size F($_{11,60}$)= 2.44, p<0.05 (based on One way Independent Measure of ANOVA).

^{a,c}differ from within group values of May at p<0.05 and <0.001 respectively (Student's '*t*' test).

Table-30: Effect of Thyroidectomy (Thx) at different stages of a Photoinduced Testicular and Body Mass cycle of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan exposed to constant 16L/8D.*

Time lag at 16L/8D	**Sham-operated**		**Thyroidectomized**	
	Body Mass (*g*±*SE*)	Testis Size (*mm*^{3}±*SE*)	Body Mass (*g*±*SE*)	Testis Size (*mm*^{3}±*SE*)
0 day (0d)	↓ (20) 38.29±0.23^{a}	1.25±0.18^{c}	(5) 38.04±0.32	1.73±0.38
1 month (1m)	(15) 39.95±0.24	27.06±2.98^{c}	0d (5) 40.04±0.33	5.29±0.76
	↓ (5) 39.86±0.37	28.11±5.66^{c}		
2 months (2m)	(10) 40.18±0.14	62.04±4.77	0d (5) 39.82±0.13	8.54±2.00
	↓(5) 40.34 ±0.31	64.75±8.47	1m (5) 40.08±0.17	8.44±0.58^{i}
3 months (3m)	(10) 39.47±0.30	64.20±5.46	0d (5) 38.80±0.27	8.80±2.53
			1m (5) 38.68±0.30	10.60±2.39^{f}
			2m (5) 39.52±0.21	9.40±2.33^{f}
4 months (4m)	(5) 39.20±0.26	36.78±5.38	0d (5) 38.66±0.24	6.67±1.56
	↓ (5) 39.60±0.30	31.69±4.03	1m (5) 38.38±0.22	5.42±1.50^{f}
			2m (5) 39.60±0.30	6.42±1.50^{f}
5 months (5m)	(5) 38.12±0.24^{a}	8.59±2.57^{c}	0d (5) 38.28±0.28	2.08±0.72
			1m(5) 38.85±0.39	2.58±1.05^{f}
			2m(5) 38.95±0.47	3.17±1.24^{f}
			4m(5) 38.55±0.27	3.58±0.92

*Values are Mean±SEM; ↓indicates point of Thx; Values in bracket denote number of birds in the group. ^{a}differs from body mass of intact birds after 2m at $p<0.05$. ^{c}differs from value of testis size of intact birds after 2m at $p<0.001$. ^{f}differs from testis size of Thx birds on day 0 at $p<0.001$.

Table –31: Effect of Thyroidectomy (Thx) at different stages of a Photoinduced Follicular and Body Mass cycle of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan exposed to constant 16L/8D.*

Time lag at 16L/8D	**Sham-operated**		**Thyroidectomized**	
	Body Mass (*g*)	Follicle Size (*mm*)	Body Mass (*g*)	Follicle Size (*mm*)
0 day (0d)	↓(16) 39.70±0.24	0.25±0.00^{c}	(4) 40.04±0.19	0.25±0.00
1 month (1m)	(12) 39.85±0.26	1.20±0.12^{c}	0d (4) 40.04±0.33	0.25±0.00
	↓ (4) 40.20±0.51	1.38±0.34		
2 months (2m)	(8) 39.78±0.24	1.55±0.10	0d (4) 39.03±0.43	0.50±0.00^{f}
	↓ (4) 38.23±0.13	1.63±0.24	1m (4) 39.38±0.43	0.50±0.00^{f}
3 months (3m)	(4) 38.94±0.18	1.51±0.53	0d (4) 38.50±0.31	0.25±0.00^{f}
	↓ (4) 38.85±0.33	0.98±0.34	1m (4) 38.58±0.25	0.25±0.00
			2m (4) 38.28±0.31	0.50±0.00^{f}
4 months (4m)	(4) 38.50±0.17^{a}	0.25±0.00^{c}	0d (4) 38.43±0.41	0.25±0.00
			1m (4) 38.35±0.25	0.25±0.00
			2m (4) 37.73±0.11	0.25±0.00
			3m (4) 38.28±0.17	0.25±0.00

*Values are Mean±SE; ↓indicates point of Thx; Values in bracket denote number of birds. ^{a}differs from body mass of sham-operated group on day 0 at $p<0.05$ level. ^{c}differs from maximum follicle size of sham-operated and Thx birds within groups at $p<0.001$. ^{f}differs from initial value of follicle size at $p<0.001$ level (Student's '*t*'test).

Table –32: Effect of Thyroidectomy (Thx) and intermittent treatments of L-Thyroxine (L-T$_4$) on Testicular Size and Body Mass of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan under constant 16L/8D daylength.*

2020 Months	Sham + Saline		Thx + L-T4		Thx + Saline	
	Body Mass (*g* ± SE)	Testis Size (*mm*^{3}± SE)	Body Mass (*g* ± SE)	Testis Size (*mm*^{3} ± SE)	Body Mass (*g* ± SE)	Testis Size (*mm*^{3} ± SE)
March	37.40±0.30	20.29±5.97	↑38.28±0.80	↑20.50±5.84	38.48±0.61	22.54±4.88
April	38.32±0.27	60.90±9.68	↓39.40±0.46	↓68.21±8.92	38.68±0.57	9.85±2.81^{d}
May	38.80±0.32	120.85±13.23	↑38.76±0.38	↑13.83±3.07	39.80±0.22	9.22±2.42^{d}
June	38.28±0.22	48.20±9.42	↓37.32±0.25	↓50.28±8.40	38.34±0.16	5.54±1.29^{e}
July	37.44±0.31^{a}	11.05±0.84	↑38.60±0.29	↑ 9.34±2.39	38.70±0.26	5.08±0.72^{e}
August	36.32±0.42^{a}	8.51±0.70	38.66±0.27	6.87±0.66	38.24±0.29	2.45±0.39^{e}

*Values are expressed as Mean±SEM; ↑ shows L-T$_4$ on (1µg/bird/day) and ↓ shows L-T$_4$ off; n=5 in each group.

^{a}differs from value of body mass in May at $p<0.05$ level. ^{d,e}differ from the within group value of Thx birds in March at $p<0.05$ and <0.01 level respectively (Student's '*t*' test).

Table –33: Effect of Thyroidectomy (Thx) and interrupted treatment of L-Thyroxine (L-T$_4$) on Follicle size and Body Mass of Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan under constant 16L/8D daylength.*

2020 Months	Sham + Saline		Thx + L-T4		Thx + Saline	
	Body Mass (*g*) ± SE	Follicle Size (*mm*) ± SE	Body Mass (*g*) ± SE	Follicle Size (*mm*) ± SE	Body Mass (*g*) ± SE	Follicle Size (*mm*) ± SE
March	38.88±0.46	1.10±0.19	↑38.18±0.54	↑1.00±0.15	38.08±0.46	1.10±0.19
April	37.92±0.32	1.46±0.13	↓38.22±0.28	↓1.46±0.13	38.68±0.39	0.50±0.00[d]
May	39.70±0.16	2.08±0.22	↑39.16±0.11	↑0.50±0.00	38.20±0.13	0.25±0.00[d]
June	38.00±0.27	1.50±0.12	↓38.34±0.29	↓1.10±0.19	37.70±0.14	0.25±0.00[e]
July	37.08±0.61[a]	0.25±0.00	↑38.02±0.56	↑0.25±0.00	37.68±0.44	0.25±0.00[e]
August	36.78±0.36[a]	0.25±0.00	37.56±0.38	0.25±0.00	37.22±0.20	0.25±0.00[e]

*Values are represented as Mean±SE; n=5 in each group. ↑shows L-T$_4$ on (1µg/bird/day), ↓shows L-T$_4$ off.

[a] differs from the value of May month at $p<0.05$ level (Student's '*t*' test). [d,e] differ from within group value of Thx birds at $p<0.05$ and 0.01 level respectively (Student's '*t*' test).

Table-34: Effect of daily Treatments of L-T_4 on Testicular Size and Body Mass of Thx Photorefractory Red-vented Bulbul, *Pycnonotus cafer stanfordi* Deignan pretreated with constant 8L/16D for 10 weeks followed by transfer to constant 16L/8D*

Months after Treatment	Intact + Saline		Thx +L-T_4		Thx+ Saline	
	Body Mass (*g*) ± SE	Testis Size (*mm*^{3}) ± SE	Body Mass (*g*) ± SE	Testis Size (mm^3) ± SE	Body Mass (*g)* ± SE	Testis Size (mm^3) ± SE
0	36.23±0.34	0.95±0.05	36.50±0.29	1.29±0.36	36.63±0.34	0.54±0.04
1	36.48±0.38	39.30±7.02^c	37.33±0.42	43.00±5.63^c	36.30±0.36	3.72±1.23
2	36.60±0.29	56.78±8.53^c	37.43±0.48	61.80±10.15^c	37.10±0.45	4.33±1.13^a

*Values are mean±SE; n=4 in each group. L-T_4= L-thyroxine (1µg/bird/day); Thx, Thyroidectomized

^{a,c}differ from value of day 0 at $p<0.05$ and <0.001 respectively (Student's '*t*' test).

Table-35: Effect of Daily Treatments of L-T_4 on Follicular Size and Body Mass of Thx Photorefractory Red -vented Bulbul, *Pycnonotus cafer stanfordi* Deignan pretreated with constant 8L/16D for 10 Weeks followed by transfer to 16L/8D*

Months after Treatments	Intact + Saline		Thx + L-T_4		Thx + Saline	
	Body Mass (*g*) ± SE	Follicle Size (*mm*) ± SE	Body Mass (*g*) ± SE	Follicle Size (*mm*) ± SE	Body Mass (*g)* ± SE	Follicle Size (*mm*) ± SE
0	36.50±0.35	0.25±0.00	36.30±0.33	0.25±0.00	35.75±0.58	0.25±0.00
1	36.78±0.49	0.88±0.24^{c}	37.18±0.38	1.13±0.24^{c}	36.53±0.47	0.25±0.00
2	36.90±0.46	1.38±0.13^{c}	37.80±0.50	1.50±0.20^{c}	36.12±0.43	0.50±0.00^{a}

*Values are mean±SE; n=4 in each group; L-T_4= L-thyroxine (1µg/bird/day); Thx, Thyroidectomized

^{a,c}differ from 0 day value at p<0.05 and p<0.001 level respectively (Student's '*t'* test).

CHAPTER – V

SUMMARY AND CONCLUSIONS

The dissertation entitled "Environmental Coorelates of Reproductive Functions in Red-Vented Bulbul (*Pycnonotus cafer stanfordi* Deignan) in Lumami, Zunhheboto District, Nagaland" presents an account of studies undertaken on various aspects of reproductive physiology and associated behavioural attributes of red-vented bulbuls, *Pycnonotus cafer stanfordi* Deignan in Lumami (26^{o}N), Zunheboto district of Nagaland State. The *P.c. stanfordi* is one of the 08 subspecies of red-vented bulbuls. It has its distribution between 23^{o}N-29^{o}N ranging from southern parts of Assam, Arunachal Pradesh, Manipur, Meghalaya, Nagaland and Tripura with further extension to Bangladesh, northern Myanmar and south-western China. The dissertation has been divided into five chapters.

Chapter-I embodies review of literature which addresses to different aspects of environmental and endocrine correlates falling within the purview of present dissertation and impart crucial role in shaping the seasonality in different physiological and behavioral attributes of birds at their different latitudinal dwellings.

Chapter-II presents an account of food resources and feeding ecology, reproduction and different aspects of physiological and behavioural strategy of red-vented bulbuls in wild and captive populations established in forested eco-habitats at Lumami (26^{o}N).

Food and Feeding Ecology*:* Red-vented bulbuls were found to consume a large variety of plant and animal food resources which flourish in the habitat linked to changes in seasons. No appreciable change was found in the quantity of food consumed by birds but seasonal preferences for quality food resources were prevalent. Birds preferred plants and/or their products at maximum during February and minimum in July-August. Conversely, intake of invertebrate food resources was lowest during February and highest during August. A large variety of plants or their products, *for example*, nectar from Indian coral trees, *Erythrina variegata* and *Erythrina indica*, banana, *Musa acuminata*, jamun, *Syzygium cumini*, guava, *Psidium quajava*, papaya, *Carica papaya*, lichi, *Lichi chinensis*, banyan, *Ficus benghalensis* figs, mulbery, *Morus nigra*, goose berry, *Phyllanthus emblica*, Cornelian cherry, *Cornus mas*, Australian cherry, *Syzygium paniculatum*, wild cherry, *Prunus aviam*, plums, *Prunus domestica*, blackshade berry, *Solanum nigrum*, ivy gourd, *Coccinia grandis*, velvetleaf fruits, *Abutilon theophrasti*, lantana, *Lantana camara* nectar, fruits and seeds, bottle brush flower, *Callistemon spps* nectar were consumed to support nutritional requirements of red-vented bulbuls. In addition, invertebrates, such as mosquitoes: *Aedes albopictus*, *Anopheles gambiae* cockroaches: *Periplanatus americana*, *Blattella germanica*, *Blattella orientalis*, ants: *Componotus spps*, *Lasius niger*, *Solenopsis spps*, grass hoppers: *Schistocerca americana*, *Phyllochoreia ramakrishnai*, *Oseudoproscopia scabra*, *Schistocera gregaria*, cicadas: cicacoidea, bush crickets: Tettigoniidae, thrips: *Winged and wingless forms*, aphids: *winged and wingless forms*, termites: *winged and wingless forms, Isoptera spps, etc* were found to add to the quality food resources across months/seasons. Seasonal diversity in the food consumed became noticeable from November-February with plant contents making the major part of food consumed (~80%). Plant food resources like guava, flower buds, nectar, Mexican sunflower seeds and invertebrates like ants, honey bees and flies made the preferred food consumed during this period. During March-May period, major part of gut-contents (<70%) were plant materials like nectar, flower buds, leaves and petals, berries, cherry fruits, and animal contents like ants, grass hoppers, thrips, aphids and termites. However, from June to October period, preference was shifted to consumption of animal food resources like spiders, gross hoppers, ants, winged-termites (~35%) and plant food nourishments like nectar, small flower buds, mixed-plant seeds, banana, papaya, guava, plums, black nightshade and velvetleaf fruits had significantly decreased in the gut-content compared with values obtained from November-June period.

Seasonal Changes in Gonads: Gonads of captive males, began developing during February-March, grew rapidly through March and April, attained peak ($125.79 \pm 13.31 mm^3$) in May, decreased significantly ($p<0.05$) during May-June and thereafter rapid decline ensued making gonads to reach regress by September. Follicular cycle closely followed testicular cycle with seasonal peak in May/June (2.70±0.14mm). The pattern of seasonal changes in gonads of captive and free living birds were temporally similar, but peak sizes of gonads (Testis: 216.26±11.12mm^3) and (Follicules:3.67±0.12mm) in May were 40% more in free living birds as compared to peak testicular (125.79±13.31mm^3) and follicular (2.70±0.14mm) sizes of captive bulbuls.

Body Mass: Body mass of captive birds was maximum in May (male: 37.75±0.47g; female: 38.22±0.29g) and minimum in July-August (35.38±0.34g; female: 36.07±0.47g). Resurgent increase in body mass of birds ensued during September-October which remained maintained until December. Body mass of free living birds paralleled changes in body mass on par with captive birds.

Feather Moult: In both the sexes, primary moult scores (PFs) varied significantly with their peak values at 30.00±1.27 in males and 31.72±0.89 in females in the month of September. In free living birds PFs moult scores were maximum in August (male: 29.33±1.22 & female: 7.50±0.56). Body feather moult (BFs) scores varied significantly in captive birds with peak values at 6.27±0.61 in males and 7.25±0.44 in females in the month of August. Similarly, significant changes in BFs moult scores of wild birds were registered with peak moult scores at 7.50±0.56 in males and 8.33±0.21 in females in August. Timings and trends for the onset and the completion of moult of PFs and BFs in both the sexes of free living and captive bulbuls were similar. It is obvious that plumage renewal process in the red-vented bulbuls is circannual at nature which expresses during post-nuptial phase.

Courtship display, Pair-bonding and Nest-site selection: Courtship display and pair-bonding could be seen starting in the mid-February and ending in September. During March/April, nomadic populations of bulbuls arrived in the study area which displayed courtship and pair-bonding occurring in quick succession followed by their settling in discrete pockets of the habitat. This feature formed peak nesting season during May-June. Courtship display and pair-bonding attributes were characterized by a minimum of 2-3 males competing to attract a female by perching closer and uttering a sequel of long, soft chirping calls of varying magnitudes. The cooing in male was followed by desperate wooing to win her response as a mate. The female responded to the preferred male by gentle flapping of her wings before him. Pair-bonding was strengthened further by grooming the feathers of each other. The follow up actions involved pair’s visit to trees one after the other and exhibit intent hopping from one branch to the other in search of a suitable nesting site. Trees holding the remains of nests fixed in the preceding season were put at preference for fixing a fresh nest. Further, before the site for placing a nest was mutually determined, birds were seen hopping around a forked-branch tilting and turning their body towards each other and exhibit motions characterizing building of a nest without any nesting material. This feature was found to be consistent amongst nesters.

Territory Establishment: Once the site for fixing a nest was determined, birds became desperate to setup a territory by earmarking an area approximating 30 square meters surrounding nesting spot. Pairs were observed defending their nesting territory against con-specific and/or hetero-specific intruders. This tendency grew stronger amidst egg-deposition and incubation. Once hatchlings appeared in the nest, birds became extra alert and profoundly aggressive in defending their territory against intruders and were often found uttering continuous alarms (*phonetically sounding as twee twee twee*) with tough gesture to combat predators tiptoeing their territory. A dry vertical branch on the tree-top was usually engaged as the view point by the male bird to guard surroundings.

Nesting sites, Shape and Sizes of Nests: Cup-shaped nests were architectured generally at the centre of a multi-forked bough of the host-plant/tree at an approximate height of 2-6 metres from the ground. Nest construction began with the framing of a ‘rim’ at the apex bordering the opening of the nest. The rim was carefully adhered to the fork using compact masses of spider webs. Thin plastic or jute ropes were often used by mates for tying nest with the host-branch. Leaf-rachis of gulmohar (*Delonix regia*), and morning glory (*Ipomoea hederifolia lutea*, *Ipomoea alba* and *Ipomoea indica*) vines were frequently used in making the nest-rim. Dry pliable leaf-rachis of gulmohar and culms of thatch grass were curved in inverted dome-shape to connect opposite sides of rim and then seal them using spider webs to create a lattice which was filled with loose interlacing of dry leaf-blades and thin stems of thatch grass growing in abundance at the study sites. Following steps included compacting of enmeshed materials starting from top towards the bottom. The bottom of the nest was generally parked at the base of the fork and knitted with long leaf-blades, flower rachis and thin stems of thatch grass. Upper side walls were knitted with smaller holes placed here and there. Both the sexes of bulbuls took part in nest construction. The female was found arranging nesting materials to weave a nest and periodic quivering to create a smooth cavity sufficient to accommodate brood and the mother. On clear sunny days, a nest could be made within 4.75±0.25 days, but when intercepted by incessant rain, the finish was delayed owing to the availability of dry nesting materials in the habitat. Recurrent insecurity posed by predators and/or hetero-specifics amidst nest construction prompted mates to abandon nest unfinished. At no point of time, two active nests could be seen parked in the same tree. The basement area of the nest measured at 117.80±2.10 cm^2 (nest with 2 eggs) and 150.34±3.15 cm^2 respectively. Mean basement area of the nest with 3 eggs was significantly more ($p<0.001$) as compared to the nest with 2 eggs only. Entry and exit routes to the nest were different and appeared to be determined prior to their positioning at the host-branch. Nesting season protracted from March/April to August/September with seasonal peak appearing around May/June. Of 300 active nests examined during the study period, 150 (50%) were found fixed in mango tree (*Mangifera indica,* from March-September), 90 (30%) in wild cherry (*Prunus avium*, during May - June), 25 (8.33%) in gulmohar (*Delonix regia,* from June-August), 20 (6.67%) in pride of India plant species, (*Lagerstroemia speciosa,* in May-June), 12 (4%) in bitter bean, stink bean or twisted cluster bean (*Parkia speciosa*, in June-July), and 3 (1%) in marigold or Mexican sunflower plants (*Tithonia diversifolia*, in June-July). Further, majority of nests were seen built lurking amongst deep foliage at the centre of tree. No nest was found built at any human raised structure.

Mating: Peak mating season clustered during May/June. The process sparked with male approaching the female and attempt to seduce her. Occasionally, female was seen responding by making advances towards male. Mutual consent culminating into mating was preceded by enticement of male by the female as a pre-requisite. During mating, female was found perching on a thin branch and by periodically slanting her body forward, she gave access to the male to mount her. At each mount, tilting his tail the male was seen transferring sperms into her genital tract. The mating was found instantly repeating for at least 2-3 times.

Egg Laying, Clutch Size and Incubation: Egg deposition began within 24-36 hours following completion of the nest. Eggs were deposited singly on consecutive days usually in the forenoon. The clutch size consisted of 2-3 eggs, oval in shape with ground pinkish colour and uneven dark red-spots all over the body. Removing an egg from the nest soon after the second or third egg was laid or adding a conspecific egg to the nest immediately after the first or second egg was deposited, did not influence the final clutch size. Incubation commenced following deposition of penultimate or ultimate egg of the clutch. Only single parent was found taking part in the incubation process. Before the start of incubation, birds were seen foraging for longer periods particularly at dawn and dusk. But, foraging timings had reduced slowly with advancement of incubation and very often, male was seen fetching a morsel of food for the incubating female, specially on days when incessant rain led to sudden drop in ambient temperature necessitating prolongation of incubation timings. Incubation was continuous during night (~18.30 hr - 4.30 hr). However, during day time parents were found leaving nest intermittently for foraging with hourly frequency at 2.65±0.90. On the average female spent 63±13% of natural lighting hours on incubating. An average on-bout and off-bout timings were at 15.54±8.50 min and 12.51±5.10 min respectively. Hatching asynchrony was observed in 69.56% cases, synchronized hatching occurred in 17.39% eggs and the remainder 13.05% eggs failed to hatch. On clear sunny days, hatching was complete within 14±0.11 days. However, when intercepted by inclement weather, incubation period was prolonged by a day or two.

Hatching and Parental Care: Eggs hatched one by one at a gap of 24-30 hr. Nestlings emerged bare bodied with eyes closed but with functional acoustic perceptions. Female continued sitting in the nest to deliver hatchlings due warmth and both sexes were found caring for nestlings against con-specific and hetero-specific intruders. Parental care began concurrent to the deposition of first egg in the nest. The stage was characterized by sounding alarms by the mates at the entry of intruder and/or predator within their territory. This tendency continued growing stronger with more eggs added to the nest. Towards completion of the clutch size, birds became extra vigilant and acquired inflexible tendency to resist unpleasant distress posed by conspecific or heterospecific encroachers of their territory. Parental care heightened with first hatchling appearing in the nest to pose obligatory feeding requirements within an hour after hatching and to continue till chicks had grown to fend for themselves. Both the sexes of bulbuls were seen fetching soft morsel of food comprising chiefly of larvae of moths and butterflies, insects (grass hoppers, aphids, thrips, ants and winged-termites *etc*), spiders, nectar, small flower buds and fruits (banana, papaya, guava, cherry, mulberry, black plums, black nightshade and velvetleaf fruits *etc*) to nourish young ones. Parents were observed nourishing chicks by turns. Soon after feeding chicks, incumbent bird was seen leaving nest sounding to his partner to take her turn for feeding. For about a week eyes remained closed, but hatchlings could smartly guess at parent’s visit to the nest as they were found instantly opening their mouth for a gape and continued begging till rewarded with a morsel of food. Gaping behaviour could be simulated in nestlings by gentle patting at the nest. Recurrent perturbations in the habitat, owing to anthropogenic activities and/or enhanced predation pressure amidst hatching, heuristically occasioned mates to heighten security concerns and consequent drop in feeding timings resulted rejection of chicks born late.

Development of Chicks: Feather papillae appeared on day 4-5 and plumages began sprouting on day 6-7. Eyes opened on day 7-8 of incubation. As the eyes opened, chicks were noticed exhibiting fear complex. The stage was characterized by nestlings start moving their wings and making noises louder than before to attract attention of their parents. Between days 10-13, nestlings became fully covered with feathers, started fluttering their wings and were seen struggling to hop out of the nest. Birds were seen strategically adjusting feeding patterns to make nestlings grow synchronously and be able to leave the nest together by day 14. However, very often, the youngest fledgling was found leaving nest delayed by a day or two. Nest was abandoned with all the chicks fledged.

Skilling the Young: Parent birds continued with nourishing fledglings and guide them to bushy sites having less frequent human interference. As the young ones grew to hopping stage, they were engaged with training protocols developed by parents. Parents generally guided them to nearby fields under moderate grass cover and sparsely growing bushes. Training was imparted daily for about an hour, preferably at dawn and dusk. The process sparked with parents catching insects for obligatory feeding of the young. This prompted young birds to instinctively hustle to grab insects for nourishing self. After about half an hour, parents guided young birds to the nearby bush and parking them there, they moved around in search of food to fend for themselves. Interestingly, during this period, parents and the young ones remained at acoustic linkages and very often one of the parents visited them with a morsel of food. Occasionally, sensing the presence of predators around, parents were seen cautioning young ones to evade from the sight of predators. Youngs were noticed instantly pushing them to a corner and sit ideal with their heads buried under wing-feathers until parents arrived to escort them. Training schedule was seen to continue for about two weeks to make young ones fully skilled for acquiring food and evade predation. During night, parents and young birds were seen roosting together in bushy trees until such times they became part of a larger foraging flock.

Murmuration Display: At Lumami (Lat. $26^{o}13'$ N, Long. $94^{0}28'$ E), bulbul murmurations were noticed occurring frequently during autumn months (late August to October). During the last week of August, distinct groups of red-vented, *Pycnonotus cafer standfordi* and olive and greyish forms of black-headed bulbuls, *Pycnonotus atriceps* or *Brachypodius melanocephalos* amassed to forage together and shared a common roost. On 12th of October 2019, a massive murmuration event was recorded in the morning hour (~6.30 am) when several hundred bulbuls (>1000 birds) foraging in discrete pockets of the habitat huddled to congregate at the communal roosting site in the hills bordering university campus in the south-west. The incidence was sparked by a single bird which began hovering over the communal roost discharging uninterrupted acoustic signal arousing others to follow, and in no time, several smaller groups joined to form a massive flock flying in synchrony above the roost. Periodically, groups were found to scatter and quickly reunite. The ever changing patterns of swirling and swooping of groups passing over and closer to their roost provided fair opportunity to the participants for intermittent exit and/or entry to the aerial show. This exercise was witnessed repeating in surges of 3-5 episodes for at least 3-5 consecutive days in the morning hours around 6.30 am lasting for about half an hour before the flock finally funneled down to the roost.

Chapter-III incorporates findings on seasonality in the photoperiodic response system of red-vented bulbul, *Pycnonotus cafer stanfordi*.

Daylength and Gonadal Growth: In NDL gonads of birds developed progressively between January and May, peaked in June which was followed by regression reaching low value in September. In December, photostimulation by 16L/8D and 20L/4D prompted rapid gonadal development. Absolute growth of gonads ensued in 3rd month which was followed by slow regression within next 3 months. Only partial gonadal enlargement occurred under constant 12L/12D. Gonads of birds exposed to constant 8L/16D photoperiod failed to develop.

Body Mass: Body mass of NDL birds measured maximum in May and minimum in August. Significant changes in body mass of 16L/8D and 20L/4D birds occurred during the course of light treatment. Body mass of birds on 12L/12D birds did not experience appreciable changes. Chronic exposure to 8L/16D photoperiod inhibited seasonal increase in body mass of birds.

Feather Moult: In NDL birds PFs and BFs renewal began in June, peaked in August which was followed by slow decline. In 16L/8D and 20L/4D groups moulting began in 04th month. PFs and BFs moult scores were at maximum in 6th month which was followed by slow decline. Groups of birds exposed to fixed durations of 12L/12D and 8L/16D daylengths did not experience feather moult.

Seasonality in Photoperiodic Response System

Light and Hypothalamo-hypophyseal-gonadal Axis: Testes began developing during January-February, grew progressively to reach seasonal maximum (105.18±11.19 mm^3) in June which was followed by decline and by August testes became smaller in size. Photostimulation by 16L/8D and 20L/4D daylengths during January led to the development of testes which peaked after 3 months followed by slow decline. Testicular growth of birds exposed to 16L/8D and 20L/4D were not significantly different from each other. Transfer of birds from NDL to 16L/8D and 20L/4D photoperiods during February and March led to maximum development of testes within 2 months. However, exposures to constant 16L/8D and 20L/4D daylengths in April and May had no effects as testes of these groups developed and regressed on par with gonads of NDL birds. Transfer of NDL birds to 16L/8D and 20L/4D during June had no effects on maximally developed testes and parallel regression ensued in testes of NDL and photostimulated groups. Exposures to 16L/8D and 20L/4D daylengths during July August and September months had no effects on regressing and/or regressed testes. However, testes developed significantly in 60% birds following transfer from NDL to 16L/8D and 20L/4D daylengths in November and 80% birds responded to photostimulation during October.

Light and Metabolic Mechanisms: In NDL body mass of birds was maximum at 38.56±0.81g in May and minimum at 35.88±0.29g in August. During July and August, mean body mass of birds were significantly less as compared to May value. Exposure of NDL birds to constant 16L/8D and 20L/4D daylengths in January and February months led to significant increase in body mass followed by regression after 2-3 months. Mean body mass of bird were significantly less as compared with the value registered after one month of light treatment. Transfer of birds from

NDL to fixed durations of 16L/8D and 20L/4D from March to November had no significant influence on body mass of birds as compared with their NDL counterparts.

Photorefractoriness Type: Continuous illumination of photorefractory birds under constant 16L/8D daylengths did not influence regressed gonads and the body mass of birds.

Chapter-IV incorporates results on light-thyroid interactions in influencing sexual and body mass cycles of red-vented bulbul, *Pycnonotus cafer stanfordi* Deignan.

Effect of Thyroidectomy on Sexual and Body Mass Cycles

Gonads: Gonads of birds began developing during February-March, attained peak in May and declined thereafter to reach seasonally low during October. Thyroidectomy performed during January inhibited seasonal increase in size of gonads compared with sham-operated birds. However, gonadal cycle with considerably small peak appeared in thyroidectomized birds during the June. Mean testicular size of thyroidectomized birds from March to July periods were significantly more as compared to values of other months.

Body Mass: Sham-operated birds exhibited maximum body mass during May which was significantly more as compared to within group values between June-August. Thyroidectomy prevented rapid decline of body mass of birds compared with sham-operated counterparts.

Effect of Thyroidectomy at different stages of photoinduced Gonadal and Body Mass Cycle

Gonads: Gonads of sham-operated birds developed maximally within 2 months of exposure to constant 16L/8D, and thereafter declined to reach low size within next 4-5 months of light treatment. Thyroidectomy suppressed the amplitude of light-induced gonadal growth in both the sexes of birds. Thyroidectomy of birds with developing and developed gonads under 16L/8D daylength led to instant regression of gonads. However, thyroidectomy performed on birds with regressing gonads did not influence gonadal regression.

Body Mass: Body mass of sham-operated group was maximum after 2 months of photostimulation by constant 16L/8D. Thyroidectomy performed at different stages of photoinduced body mass cycle failed in influencing body mass of both the sexes of red-vented bulbuls.

Effect of thyroidectomy and intermittent replacement with L-T_4 on development of Photorefractoriness: Gonads of sham-operated birds exposed to constant 16L/8D daylength during March developed rapidly, peaked in May and thereafter declined to reach a low value in August. Thyroidectomy during March suppressed developing gonads of birds following transfer to constant 16L/8D. Further, intermittent treatments of L-T_4 at 1 µg/bird in thyroidectomised birds led to recovery of gonads only to the level of sham-operated group in contemporary months. Intermittent withdrawal of L-T_4 injections resulted in rapid decrease of gonads to the level of thyroidectomised group receiving saline only. L-T_4 treatments in thyroidectomised birds during July-August (4 months after exposure to 16L/8D) failed in preventing gonadal regression. Mean body mass of sham-operated birds measured maximum at 2 months of exposure to constant 16L/8D photoperiod which measured significantly more as compared to mean body mass of birds after 4-5 months of exposure to 16L/8D. Thyroidectomy and/or intermittent treatments with L-T_4 at 1 µg/bird failed in influencing body mass of thyroidectomized birds.

Short day induced recovery of photosensitivity in intact and thyroidectomised birds: Gonads of photorefractory birds pre-treated with constant 8L/16 daylength for 10 weeks developed significantly following transfer to constant 16L/8D daylength. Small but significant gonadal enlargement ensued in thyroidectomised photorefractory birds pre-exposed to fixed duration of 8L/16D following transfer to constant 16L/8D. Treatment of thyroidectomised birds with L-T_4 at 1µg/bird led to the development of gonads on par with intact birds.

Conclusions

- Red-vented bulbul, *Pycnonotus cafer stanfordi* is basically a frugivorous bird but exhibits preferences for animal food resources during post-breeding season to cope with increased calorie requirements towards parental care and recoupment of energy stocks depleted during intense breeding activities and feather renewal occurring concurrently.

- Gonads and body mass of birds were symmetrically positions to solar cycle and exhibited distinct seasonality with peaks temporally coinciding with longest days during May/June. Post-nuptial decline of gonads was accompanied with moulting of feathers. These results suggest that red-vented bulbuls are equipped with the mechanisms to economize their energy resources across the season. Temporal linkages of gonadal and body mass cycles may denote that two cycles are engendered by some common physical and/or physiological mechanisms. Significantly (40%) curtailed gonadal development in birds under captive conditions may be attributed to suppressed secretion of GnRH under the captivity stress.

- Critical daylength for development of gonads and body mass appeared to be around 11 hr/day. However, these indices expressed fully only under daylengths around summer solstice (13hr, 30min). Post-nuptial decline in gonads and body mass of birds occurred due to development of absolute photorefractoriness.

- Birds are habituated to construct cup-shaped nest using locally available materials. Entry and exit routes to the nest were different and were determined prior to the placing of nest. Nesting season protracted from spring equinox to autumnal equinox with peak clustering around summer solstice. Nesting was accompanied by host of activities which included courtship, pair-bonding and mating followed by deposition of 2-3 pinkish color eggs with uneven dark spots all over body, incubation and post-fledging skilling of young birds. Egg manipulation during different stages of deposition did not influence the clutch size. On the average nest-construction was complete within 4.5±0.25 days. Basement area of nest differed with the clutch size and was significantly less for nests with 2 eggs compared with nests with 3 eggs. Incubation period lasted for 14±0.11 days. Fledglings grew to full-fledged skilled individuals within 2 weeks. Fledging rate was in excess of 60%.

- Photoinduced increases in gonads and body mass of red-vented bulbuls were not different under constant 16L8D and 20L/4D daylengths. Developed gonads and body mass of birds were sustained for few weeks and then regressed due development of absolute photorefractoriness. Photoperiodic system of red-vented bulbul exhibited distinct seasonality in response to photostimulation. Neuroendocrine–gonadal and neuroendocrine-metabolic axes were fully responsive to long daylengths around winter solstice. These axes continued losing photosensitivity with progressive increases in daylength from winter solstice to summer solstice paving way to development of photorefractoriness–gonadal and metabolic- around summer solstice. Progressive decreases in daylengths from summer solstice to winter solstice became instrumental in gradual dissipation of photorefractoriness and resumption of photosensitivity.

- Thyroidectomy inhibited gonadal development but had no influence on body mass of birds. The influence of thyroidectomy on gonads and body mass of birds depended on the sexual stage at the time of surgery. Similarly, influence of L-thyroxine at daily dose of 1µg/bird/day depended upon the sexual phase of the birds at treatment. Further short daylength terminated photorefractoriness in intact and thyroidectomized birds alike.

All credentials incorporated in the current findings point to the fact that photorefractory birds are thyrorefractory as well. It remains to be ascertained whether photorefreactoriness leads to thyrorefractoriness or *vice-versa*.

The murmuration recorded in the inter-specific flocks of red-vented and black-headed bulbuls is a rare aerial exercise not described for bulbuls ever before. It remains to be established whether this aerial show is areal or it characterizes inherent defense strategy of these Pycnonotids against common predators.

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