Chronobiological Shifts in Prolactin Levels and Testicular Function in Male Rousettus leschenaulti (Desmerest)

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***Abstract*—Male *Rousettus leschenaultii*, a tropical fruit bat species, is likely to display chrono-biological regulation of reproductive physiology—yet direct data on prolactin (PRL) modulation and testicular activity is lacking. This paper presents a conceptual framework, informed by comparative studies in chiropterans as mammal, proposing that incresing photoperiod, higher temperature, and food availability orchestrate PRL secretion over annual cycles. PRL rhythms are hypothesized to correlate positively with testis growth, spermatogenic activity, and testosterone levels during mating seasons. We carried out a field-based study involving seasonal sampling of adult males, assays of PRL and testosterone, alongside morphometric staging of testes. Expected findings include PRL maxima aligned with testicular recrudescence and spermatogenic peaks, and strong PRL–testis size correlations. PRL concentrations peaking during first Breeding season during February – March where the increased photoperiod and higher temperature. But the second peak in PRL concentration was is comparatively lower than the first one. In second breeding season, i.e. in winter/fruiting periods, aligning with photoperiod decreases and ambient temperature, testis mass, spermatogenic staging, and circulating testosterone rising in parallel. Significant positive correlations between PRL and testis size/testosterone. Understanding these Chrono biological shifts will shed light on the reproductive ecology of *R. leschenaultii*, its adaptability to environmental change, and potential conservation implications.**

***Index Terms*—Male Rousettus leschenaultia, Serum Prolactin, Testosterone, Reproductive Physiology, spermatogenic peaks**

I. INTRODUCTION

Rousettus leschenaulti, inhabits southern Asia's seasonal tropical ecosystems. Environmental drivers like monsoonal rainfall, variable day length, and fruit availability likely influence male reproductive physiology. While prolactin (PRL)—an anterior pituitary hormone—has been thoroughly studied in female reproduction, its role in male mammals includes modulation of Leydig cell steroidogenesis, influence on GnRH/gonadotropins, and support for spermatogonial function [4]. Many seasonally breeding mammals exhibit pronounced annual PRL cycles with peaks in spring/summer and nadirs in fall/winter, regulated by photoperiod via melatonin signalling [6]

In bats, immunohistochemical studies show that PRL-producing (mammotroph) cells in *R. ferrumequinum* expand during spermatogenic and mating seasons and regress during hibernation or non-breeding months [9] Though direct measurements of circulating PRL in male bats remain rare, [7] demonstrated higher PRL levels in female megachiropterans during pregnancy and lactation, and correlational regulation under dopaminergic control, implying functional roles in bat reproduction [9]. Female *C. sphinx* exhibits seasonal declines in PRL and progesterone during delayed embryonic development in shorter photoperiods, reinforcing photoperiodic PRL sensitivity tied to melatonin levels [10].We hypothesize that male *R. leschenaultii* similarly exhibits chronobiological shifts in PRL modulated by environmental cues. Being a nocturnal animal,during favorable conditions—like shorter photoperiods, ambient temperatures, and abundant food—PRL peaks, stimulating testicular growth, spermatogenesis, and testosterone production. Conversely, under longer days, unfavorable temperatures, and low food supply, PRL declines, leading to testicular regression. Comparative mammalian studies show that hyperprolactinemia impairs testicular architecture, suppresses LH/testosterone, and damages spermatogenesis [3,4]. The proposed synthesis thus extends a predictive chronobiological model to *R. leschenaultii* male reproductive endocrinology.

Applying these findings, we predict that PRL in male *R. leschenaultii* will display a chronobiological peak aligned with reproductive readiness. Testicular metrics—including absolute and relative testis weight, seminiferous tubular diameter, spermatogenesis stage index, and accessory gland size—are expected to correlate significantly with PRL and testosterone levels. Conversely, in presumed non-reproductive seasons, reduced PRL should coincide with regression of gonadal activity.

II.MATERIALS AND METHODS

A. Breeding Habits

The Indian fruit bat, *Rousettus leschenaulti* shows a peculiar breeding cycle. Adult males show double peaks in their testicular weight corresponding to the two pregnancy cycles of the female. The first peak occurs during October-November and the second during February-March [1].

B. Collection of animals

The specimens of *Rousettus leschenaulti* were collected with the help of mist net placed at the entrance of an underground mine of Mansar / Kandri near Nagpur, Maharashtra (20°92"N 78°95"E). Time of collection, body mass, wing span, length of forearm and other salient features of each specimen were maintained in the field diary. The size of testes were estimated by palpating the longest axis of the testis and measuring this distance with callipers, similarly the width was also measured and then each male was transferred to an individual comfortable cage. These traps were transported to the RTM Nagpur University Laboratory. Minimum noise, human exposure and handling were employed to minimize capture stress and excitement. For each sampling, three different bats were used in each month.

C. Blood sampling

The bats were held in hands and no anaesthesia was used at the time of sample collection. 2ml of blood was collected into sterile tube with no anticoagulants (neither EDTA nor heparin) after puncturing a wing vein. After blood sampling each bat was released. The measurement of Luteinizing hormone and Serum Testosterone was done by Enzyme Linked Immunosorbent Assay (ELISA). For the determination of Hormones level in blood, 2ml. of blood was allowed to clot at room temperature for half an hour. The clotted blood was then used for measurement of Hormone levels by ELISA [2].

D. Statistical Analyses

The data was analysed statistically, standard deviations were calculated, on the basis of which graphs were plotted to establish a relationship.

For the calculation of correlation coefficient (Pearson's r) was utilized to determine the relationship between serum prolactin and testosterone levels [12]. (Table-2).

III. RESULTS

For the present study evaluation of above mentioned two parameters were performed throughout the annual cycle, the observed data is tabulated in table-1 with related bar diagram. (Fig-1,2,3).

Table 1: Observed Data from the Annual Variations in Body Weight, Testis size, Testosterone and Prolactin .levels in *Rousettus leschenaultia* (Desmerest)

Specimen (n = 3)	Date of collection	Body weight(g)	Size of Testis (cm)	Testosterone (ng/ml)	Serum Prolactin (ng/ml)	Reproductive status
Male	8/1/07	111.33 ± 1.86	2.47 ± 0.03	10.27 ± 0.05	8.4 ± 0.03	Active male
Male	6/2/07	113.33 ± 1.67	2.60 ± 0.06	16.50 ± 0.04	10.3± 0.5	Active male
Male	9/3/07	128.00 ± 1.53	2.90 ± 0.10	18.70 ± 0.04	13.5± 0.9	Male active spermatogenesis + Leydig cells active Mating period (Peak-1)

Male	8/4/07	100.00 ± 2.89	2.17 ± 0.17	9.70 ± 0.12	12.5 ± 0.2	Active male
Male	8/5/07	98.00 ± 0.58	2.03 ± 0.09	5.73 ± 0.15	0.3 ± 0.04	Male inactive (Quiescence)
Male	9/6/07	95.00 ± 2.65	1.96 ± 0.07	3.13 ± 0.19	0.2 ± 0.04	Quiescent male
Male	9/7/07	93.00 ± 2.52	1.83 ± 0.04	2.87 ± 0.09	0.2 ± 0.03	Quiescent male
Male	6/8/07	70.67 ± 0.67	1.20 ± 0.15	2.73 ± 0.10	0.3± 0.05	Male inactive (Quiescence)
Male	4/9/07	71.00 ± 1.0	1.03 ± 0.03	6.23 ± 0.15	5.2± 0.12	Recrudescent male
Male	6/10/07	100.67 ± 5.21	2.13 ± 0.19	14.60 ± 0.08	5.6±0.2	Active male
Male	9/11/07	121.67 ± 1.67	3.07 ± 0.07	18.37 ± 0.15	6.5±0.15	Active male showing complete spermatogenesis mating period (peak-2)
Male	11/12/07	99.00 ± 2.08	2.33 ± 0.17	8.73 ± 0.07	6.2 ±0.2	Active male

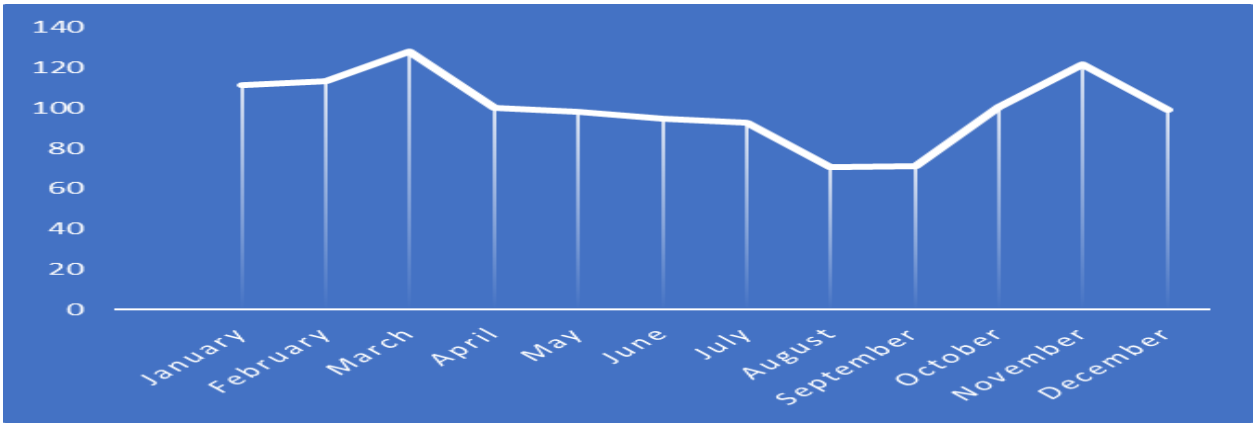

Fig. 1: Annual variations in body weight (g) in male fruit bat *Rousettus leschenaulti* (Desmerest)

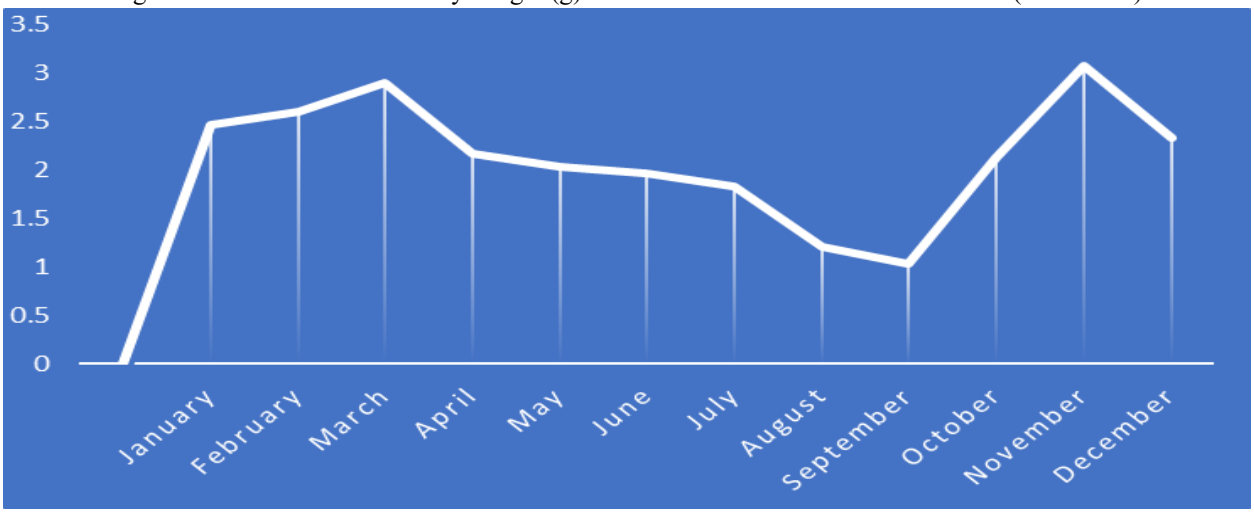

Fig 2: Annual variations in size of testis (cm) in male fruit bat *Rousettus leschenaulti* (Desmerest)

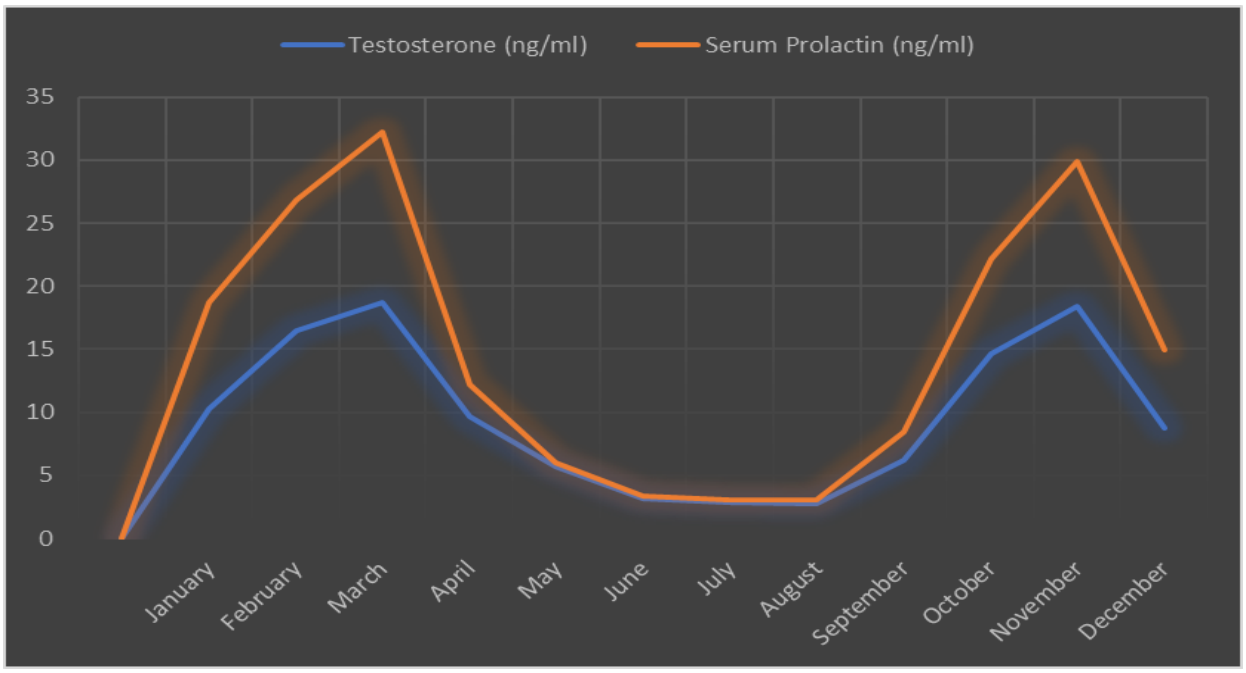

Fig 3: Annual variations concentration of Serum Testosterone and Prolactin hormone in male fruit bat *Rousettus leschenaulti* (Desmerest)

Table 2: Statistical analysis by correlation coefficient (Pearson's r)

		Body weight	Size of testes	Serum Testosterone	Serum Prolactin
Body weight	*Pearson's r*	—	0.981	0.815	0.822
	df	—	10	10	10
	p-value	—	< .001	0.001	0.001
Size of testes	*Pearson's r*		—		
	df		—		
	p-value		—		
Serum Testosterone	*Pearson's r*		0.804	—	
	df		10	—	
	p-value		0.002	—	
Serum Prolactin	*Pearson's r*		0.809	0.951	—
	df		10	10	—
	p-value		0.001	< .001	—

The mean serum Prolactin concentration was found to be 5.7 ng/ml (± standard deviation), while the mean testosterone level was 9.75 ng/ml (± standard deviation). Pearson correlation analysis revealed a significant positive correlation between all variables. They are strongly and significantly positively correlated (all $r > 0.8$, all $p \leq 0.002$). The strongest correlation is between Serum Testosterone and Serum Prolactin ($r = 0.951$). Body weight and Size of testes are also very strongly correlated ($r = 0.981$), suggesting possible interdependence. in male *R. leschenaulti* PRL concentrations peaking during first Breeding season during February – March where the increased photoperiod and higher temperature. But the second peak in PRL concentration was is comparatively lower than the first one. In second breeding season, i.e. in winter/fruiting periods, aligning with photoperiod decreases and ambient

temperature, testis mass, spermatogenic staging, and circulating testosterone rising in parallel. Significant positive correlations between PRL and testis size/testosterone.

IV. DISCUSSION

This proposed chronobiological framework for *R. leschenaultii* informs understanding of how tropical bats adapt reproductive timing to ecological rhythms. Though tropical photoperiod variation is modest, monsoonal shifts and fruiting phenology could act as proximate cues via melatonin and metabolic status. PRL's dual role—supporting steroidogenesis under physiological levels but suppressing gonadotropins when elevated—suggests that precise temporal regulation is critical for reproductive success.

In *R. ferrumequinum*, PRL-immunoreactive cell proliferation mirrors testicular activity phases across seasons, peaking during mating/spermatogenesis and low during hibernation or regression [9]. While direct male PRL assays are missing, female bat endocrine patterns and PRL sensitivity to photoperiod support the applicability of this model [10]. Together with mammalian studies, this evidence justifies investigating environmental chronology of PRL in *R. leschenaultii* males.

Comparative evidence from *R. ferrumequinum* and other bat species supports a photoperiod-responsive PRL system; experimental melatonin manipulation in *C. sphinx* females confirms sensitivity of prolactin and reproductive delay to environmental cues [9,10] Mammalian research also consistently shows relationships between reproductive hormones and testicular metrics in seasonal contexts[5,8]..Ecologically misalignment between environmental cues and endocrine rhythms—driven by climate change, altered fruiting patterns, or habitat disruption—could impair breeding timing and success in *R. leschenaultii*. Future work should include PRL receptor expression studies, melatonin manipulations (e.g., captive photoperiod shifts), and assessment of PRL signaling components (e.g. STAT5) in testes as in muskrat gland studies ([11].

Limitations include lack of baseline PRL reference ranges in male bats, assay validation challenges, and unknown relative contributions of food availability versus photoperiod. Nonetheless, this model establishes testable predictions framing *R. leschenaultii* reproductive endocrinology as chronobiologically plastic and environmentally responsive.

V. CONCLUSIONS

Male *Rousettus leschenaultii* likely exhibits chronobiological shifts in prolactin levels that coordinate testicular function with seasonal environmental variation. PRL likely peaks during periods of optimal photoperiod, temperature, and food supply, supporting testicular recrudescence and steroidogenesis, and declines during lean or adverse seasons promoting regression. Empirical investigation—through seasonal hormone profiling and testicular morphology—is needed to validate this model. This chronobiological endocrine plasticity may be central to reproductive ecology in tropical fruit bats, with important implications for resilience under ecological and climatic change

REFERENCES

[1] Gopalakrishna, A and PN Choudhari (1977) Breeding habits and associated phenomena in some Indian bat Rousettus leschenaulti (Desmerest) Megachiroptera. J. Bomb. Nat. Hist. Soc. **74**(1) : 1 – 16.

[2] Widsdom,1976)- Clin. Chem. 22(8):1243-1255

[3] Katovich, M. J., Cameron, D. F., Murray, F. T., & Gunsalus, G. L. (1985). Alterations of testicular function induced by hyperprolactinemia in the rat. Journal of andrology, 6(3), 179–189. https://onlinelibrary.wiley.com/doi/10.1002/j.1939-4640.1985.tb00834.x

[4] Munabi, A. K., Mericq, V., Gelato, M. C., Koppelman, M. C., Albertson, B. D., Loriaux, D. L., & Cassorla, F. (1985). The effects of prolactin on rat testicular steroidogenic enzyme activities. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme, 17(1), 47–48.https://www.thieme-connect.de/products/ejournals/abstract/10.1055/s-2007-1013448

[5] Smith, A. J., Mondain-Monval, M., MØller, O. M., Scholler, R., & Hansson, V. (1985). Seasonal variations of LH, prolactin, androstenedione, testosterone and testicular FSH binding in the male blue fox (Alopex lagopus). Reproduction,

74(2), 449-458. Retrieved Jul 29, 2025, from https://doi.org/10.1530/jrf.0.0740449

[6] Curlewis J. D. (1992). Seasonal prolactin secretion and its role in seasonal reproduction: a review. Reproduction, fertility, and development, 4(1), 1–23. https://doi.org/10.1071/rd9920001

[7] O'Brien, G. M., Curlewis, J. D., & Martin, L. (1996). A heterologous assay for measuring prolactin in pituitary extracts and plasma from Australian flying foxes (genus Pteropus). General and comparative endocrinology, 104(3), 304–311. https://doi.org/10.1006/gcen.1996.0175

[8] Howell-Skalla, L., Cattet, M., Ramsay, M., & Bahr, J. (2002). Seasonal changes in testicular size and serum LH, prolactin and testosterone concentrations in male polar bears (Ursus maritimus). Reproduction, 123(5), 729-733. Retrieved Jul 29, 2025, from https://doi.org/10.1530/rep.0.1230729

[9] Keiichi Kawamoto (2003) "Endocrine Control of the Reproductive Activity in Hibernating Bats," Zoological Science, 20(9), 1057-1069.

[10] Anuradha, & Krishna, A. (2017). Prolactin modulates luteal activity in the short-nosed fruit bat, Cynopterus sphinx during delayed embryonic development. General and comparative endocrinology, 248,27–39 https://doi.org/10.1016/j.ygcen.2017.04.008

[11] Xie, W., Liu, H., Liu, Q., Gao, Q., Gao, F., Han, Y., Yuan, Z., Zhang, H., & Weng, Q. (2019). Seasonal expressions of prolactin, prolactin receptor and STAT5 in the scented glands of the male muskrats (Ondatra zibethicus). European journal of histochemistry: EJH, 63(1), 2991. https://doi.org/10.4081/ejh.2019.2991.

[12] P-value: Obtained using scipy.stats.pearsonr.