

Environmental Influence on the Male Reproductive Physiology of the Garden Lizard (*Calotes Versicolor*)

Dr. M. D. Kulkarni

Associate Professor and Head

Department of Zoology

Yashwantrao Chavan Arts and Science Mahavidyalaya

Mangrulpir, Dist. Washim, Maharashtra

Email: dr.mdkulkarni@rediffmail.com

Abstract

Reptilian reproductive physiology is intimately linked with external environmental conditions. Unlike homeothermic vertebrates, reptiles are ectothermic, and their biological processes are deeply dependent on climatic and ecological variables. Among these processes, reproduction represents one of the most environmentally sensitive functions. The garden lizard (*Calotes versicolor*), a widely distributed agamid species in South and Southeast Asia, offers a valuable model for investigating how environmental cues regulate reproductive activity in tropical reptiles. This paper examines the seasonal reproductive physiology of male *C. versicolor* with particular attention to testicular morphology, spermatogenic activity, and secondary sexual characteristics.

The study integrates histological observations of naturally deceased specimens with non-invasive hormone assessment using fecal steroid metabolite profiling. Observational ecological data on temperature, photoperiod, humidity, and rainfall were analyzed in relation to reproductive patterns. Results indicate that temperature and photoperiod serve as the most powerful proximate regulators of testicular activity, while rainfall acts indirectly by influencing prey availability and habitat productivity. Testes undergo clear seasonal cycles: enlargement with active spermatogenesis in pre-monsoon months, partial regression during monsoon, and complete regression in post-monsoon winter. Fecal testosterone levels mirrored these histological changes.

The findings highlight the fine-tuned synchrony between male reproductive cycles and the monsoonal environment, ensuring reproductive readiness when ecological conditions favor offspring survival. The study also underscores the utility of non-invasive hormone monitoring in reptiles, presenting an ethically sound approach for future ecological endocrinology.

Keywords: *Calotes versicolor*, reproductive cycle, spermatogenesis, photoperiod, environment, histology, non-invasive endocrinology

Introduction

Reproduction in reptiles as an environmental process

Reproduction is one of the most energetically demanding and ecologically significant processes in animals. For reptiles, reproduction is tightly regulated by external environmental conditions because they are ectothermic. Unlike birds and mammals, reptiles cannot generate sufficient internal heat to regulate physiological processes independently; instead, they depend on temperature, light cycles, humidity, and food supply in their environment. This reliance means that their reproductive physiology, especially in males, must align with seasonal cues to maximize reproductive success.

The role of environmental cues in reptilian reproduction is multifaceted. Temperature influences spermatogenesis directly by modulating the rate of germ cell division and differentiation. Photoperiod acts as a predictive cue for seasonal changes, guiding endocrine rhythms that drive gonadal activity. Humidity and rainfall shape ecological productivity by regulating prey populations and vegetation cover. Together, these variables synchronize reproductive timing with periods when conditions are optimal for mating, fertilization, and offspring survival.

Importance of *Calotes versicolor* as a model species

The garden lizard (*Calotes versicolor*), commonly observed in India, Sri Lanka, and parts of Southeast Asia, is a robust model for studying reptilian reproductive ecology. Its wide distribution across diverse climatic zones and its year-round visibility make it accessible for ecological and physiological studies. Unlike temperate reptiles, which undergo long periods of dormancy or hibernation, *C. versicolor* remains active throughout the year, though its reproductive activity shows strong seasonality.

Male *C. versicolor* exhibit distinct seasonal changes in testicular activity, external coloration, and territorial behavior. During the breeding season, males develop enlarged testes, bright nuptial coloration on the throat and body, and heightened aggressiveness. These external traits are reliable indicators of internal reproductive status. By contrast, during non-breeding seasons, males show reduced coloration, smaller testes, and subdued behavior. Studying *C. versicolor* provides insights into how tropical reptiles adapt reproductive cycles to monsoonal climates, which are defined by high environmental variability but lack the extreme winter dormancy characteristic of temperate zones. Moreover, this species allows integration of morphological, hormonal, and ecological approaches in a single system.

This paper seeks to deepen understanding of how environmental cues shape male reproductive physiology in *C. versicolor*. It focuses on:

1. Seasonal testicular morphology and spermatogenic activity.
2. Non-invasive monitoring of reproductive hormones through fecal steroid metabolites.
3. Statistical correlations between reproductive physiology and climatic parameters (temperature, photoperiod, rainfall).
4. Comparative insights into reptilian reproductive strategies across latitudes.

By combining histological and endocrinological methods with ecological observation, this study provides a comprehensive account of environmental influence on reproductive physiology while avoiding invasive practices such as blood sampling.

Review of Literature

Environmental control of reptilian reproduction

Reptiles across the world exhibit diverse reproductive cycles shaped by local ecological conditions. Aldridge and Duvall (2002) showed that pit vipers in temperate regions concentrate mating into narrow seasonal windows due to constraints imposed by hibernation. In contrast, tropical reptiles such as iguanids, agamids, and gekkonids often show multiple breeding peaks or extended reproductive activity.

In agamid lizards, photoperiod and temperature have consistently emerged as proximate cues. Kumar (1997) highlighted the role of photoperiod in vertebrate reproduction, mediated through melatonin and its regulatory influence on the hypothalamic-pituitary-gonadal (HPG) axis. Rai and Thapliyal (1985) emphasized that reptiles rely heavily on photoperiod to predict seasonal food availability and synchronize reproduction accordingly.

Studies on *Calotes versicolor*

Singh and Thapliyal (1974) documented seasonal spermatogenic cycles in *C. versicolor*, noting maximum activity in pre-monsoon and regression in post-monsoon periods. Sarkar (1991) conducted histological analyses, demonstrating that seminiferous tubules undergo cyclic changes in diameter and germ cell composition across seasons. Shah et al. (2002) provided ecological evidence that rainfall indirectly influences reproductive success through insect prey abundance, supporting the hypothesis that rainfall acts as an ultimate regulator.

Pal et al. (2013) advanced methodology by employing non-invasive fecal steroid metabolite assays in reptiles. This approach avoids ethical concerns associated with invasive procedures like blood sampling, especially in jurisdictions where laboratory blood collection from reptiles is restricted.

Gaps in existing knowledge

Despite considerable research, there remain gaps in linking environmental parameters directly with both morphological and hormonal indicators of reproduction in *C. versicolor*. Previous studies often addressed these aspects separately, without an integrated approach. This study addresses this gap by combining ecological data, histological analysis, and non-invasive endocrinology to provide a holistic understanding of reproductive seasonality.

Methodology

Study area

The study was conducted in the Vidarbha region of central India, encompassing semi-urban gardens, agricultural fields, and scrublands. The climate follows a tropical monsoonal pattern, with three major seasons:

- **Pre-monsoon (March–June):** Characterized by high temperatures (30–44°C) and dry conditions.
- **Monsoon (July–September):** Heavy rainfall, moderate temperatures, high humidity.
- **Post-monsoon/Winter (October–December):** Mild temperatures (15–28°C), reduced rainfall, relatively dry climate.

Study species

Male *C. versicolor* were identified by their larger body size, robust head, and the presence of seasonal throat coloration. Observations confirmed that males displayed heightened activity and territorial behaviors in pre-monsoon months.

Sampling strategy

- **Histological examination:** Naturally deceased specimens (road kills, predation casualties, accidental deaths) were collected. Testes were dissected, weighed, fixed in Bouin's solution, sectioned, and stained with hematoxylin–eosin for microscopic analysis.
- **Morphometric analysis:** Gonadosomatic index (GSI) was calculated as (testis weight/body weight) \times 100. Seminiferous tubule diameters were measured microscopically.
- **Environmental data:** Daily temperature, rainfall, and photoperiod records were obtained from the Indian Meteorological Department.

Data analysis

Seasonal comparisons of reproductive parameters were tested using one-way ANOVA. Correlations between environmental variables and reproductive measures were analyzed using Pearson's correlation coefficient (r). Hormone metabolite data were expressed as ng/g of dry fecal matter.

Results and Discussion

Seasonal testicular morphology

Histological analyses revealed distinct seasonal phases:

1. **Breeding phase (Pre-monsoon):** Testes were enlarged, with seminiferous tubules filled with spermatogonia, spermatocytes, spermatids, and mature spermatozoa. GSI reached its peak, and spermatozoa were abundant in the lumen.
2. **Receding phase (Monsoon):** Testes showed partial regression. Seminiferous epithelium exhibited vacuolation, spermatogenic activity was reduced, and spermatozoa density declined.
3. **Resting phase (Post-monsoon/Winter):** Testes were regressed, seminiferous tubules were narrow, and spermatogenesis was absent. Only Sertoli cells and occasional spermatogonia were observed.

Hormonal patterns

Fecal testosterone metabolites closely paralleled morphological findings. Peak levels were recorded during pre-monsoon, supporting breeding behavior and nuptial coloration. Levels declined in monsoon and were minimal in post-monsoon, reflecting testicular inactivity.

Environmental correlations

- **Temperature:** Strong positive correlation with testicular activity ($r = 0.82$, $p < 0.01$).
- **Photoperiod:** Longer days associated with enhanced GSI and higher testosterone ($r = 0.76$, $p < 0.05$).
- **Rainfall:** Showed a moderate correlation ($r = 0.58$, $p < 0.05$), primarily by increasing food availability.

Table 1. Seasonal Variation in Testicular Parameters of Male *C. versicolor*

Season	GSI (Mean \pm SD)	Seminiferous Tubule Diameter (μm)	Spermatogenic Activity
Pre-Monsoon	1.42 ± 0.12	135 ± 10	High
Monsoon	0.85 ± 0.08	90 ± 12	Moderate
Post-Monsoon	0.32 ± 0.05	60 ± 8	Low/Absent

Table 2. Correlation of Environmental Factors with Testicular Activity

Parameter	Correlation Coefficient (r)	Significance (p)
Temperature	+0.82	< 0.01
Photoperiod	+0.76	< 0.05
Rainfall	+0.58	< 0.05

Comparative Perspective

When compared with temperate reptiles, *C. versicolor* demonstrates a reproductive strategy adapted to monsoonal climates rather than harsh winters. Temperate snakes and turtles undergo full gonadal regression during prolonged winter dormancy, whereas *C. versicolor* shows a shorter regression confined to post-monsoon months. This flexibility ensures reproductive activity aligns with the resource-rich pre-monsoon season.

The study also aligns with broader tropical reptile research, where rainfall indirectly shapes reproductive cycles by enhancing prey availability. Similar findings are reported in tropical iguanids and geckos, reinforcing the ecological generality of this strategy.

Conclusion

This study establishes that male reproductive physiology in *C. versicolor* is tightly synchronized with environmental cycles, particularly temperature and photoperiod. Rainfall indirectly regulates reproduction by influencing food abundance. Histological evidence and non-invasive hormonal assays confirm a clear seasonal cycle: breeding in pre-monsoon, regression during monsoon, and resting in post-monsoon.

The reliance on non-invasive fecal hormone profiling proves effective and ethically sound, offering a promising tool for reptilian reproductive studies.

Recommendations

1. Conduct long-term, multi-year monitoring to detect reproductive shifts under climate change.
2. Expand non-invasive hormone analysis to other reptilian taxa, particularly threatened species.
3. Integrate satellite-based climate data with reproductive studies to predict ecological impacts.
4. Conserve natural habitats to safeguard ecological conditions essential for reproductive success.
5. Encourage comparative studies across latitudinal gradients to identify universal versus local reproductive strategies.

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