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Seasonal Variation in Gonads of Bufo Viridis in Rajasthan

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Abstract

Amphibian populations have declined significantly in recent years due to habitat depletion and poor management of agro ecosystems. A change in climate, habitat, etc., has an impact on amphibian's capacity to reproduce. Similar to other amphibians, Bufo viridis are seasonal breeders, meaning that most of the year is spent in reproductive activity, with the gonads remaining essentially dormant for the remainder of the year. The purpose of the study was to determine the seasonal variations in the activity of the ovaries and testes of mature Bufo viridis inhabitants of the Rajasthan area.

Keywords

Bufo viridis, population, gonad, ecosystem, and amphibian.

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INTRODUCTION:

The main geographical feature of Rajasthan, the Aravalli range, divides the state diagonally into a 2/5 semi-arid zone in the east and a 3/5 desert region in the west. Rajasthan has three distinct seasons based on its climate: summer (March to mid-June), rainy (mid-June to mid-September), and winter (October to mid-February) (2). East of the Aravallis is home to a large population of amphibians. The districts of Kota, Braran, Jhalawar, Chittorgarh, Bhilwara, Rajsamand, Udaipur, Banswara, Dungarpur, and Sirohi are home to a multitude of species. (3)

It is common for, like other amphibians, to only engage in breeding at specific seasons of the year, with their gonads staying largely dormant for the remainder of the year. Gonads have been seen to remain active all year round in certain anurans, though (8). The Arid Zone has significantly higher maximum temperatures than the mean, with most of the state experiencing extremes in temperature. The mean summertime maximum temperature falls between 40.5°C and 42°C (9).

The climates of this country fluctuate in different regions, with the north experiencing Alpine conditions, the south experiencing tropical climates, and the east experiencing a consistently moist and actually cooler environment, while Rajasthan in the west experiences scorching and dry desert conditions (12). For all living creatures, reproduction is the most basic function. Reproduction is necessary for a species to survive even in the absence of the other so-called distinctive traits like irritability, metabolism, etc.

The earliest vertebrates to adapt to life on land are amphibians. Ponds, grassy areas, swampy ditches, gardens, etc. are home to Bufo viridis. In the winter, frogs and toads hibernate, and in the summer, they aestivate. Despite the lack of published data on the community structure of amphibians in Indian ecosystems, mismanagement of agricultural ecosystems and habitat depletion have led to a numerical decline in amphibian populations in recent years (5). The Indian government has outlawed the export of frog legs based on this supposition. Changes in climate, habitat, etc., had an impact on amphibians' capacity to reproduce (15). Spermatogenesis is a dynamic process characterized by multiple stages, typically divided into three main events: spermatocytogenesis, where spermatogonial mother cells multiply to form spermatogonial populations; meiotic division, during which diploid primordial germ cells divide to produce haploid spermatids; and spermiogenesis, the final transformation of spermatids into spermatozoa.

The process of spermatogenesis is a dynamic one that involves several stages and is generally classified into three main events: meiotic division, which is the division of diploid primordial germ cells to produce haploid spermatids, spermiogenesis, which is the last stage in which spermatids become spermatozoa, and spermatocytogenesis, which is the multiplication of spermatogonial mother cells to form spermatogonial populations.

Several significant changes occur, such as the development and differentiation of germ cells, the growth of protogerminant cells, a decrease in the number of chromosomes, the transformation of haploid products into spermatids or spermatozoa, and maturation processes after metamorphosis.

Spermatogenesis in this discontinuous species takes place after the breeding season in late summer, peaking in June. Diverse races within the same species of Bufo viridis that inhabit disparate climatic circumstances have been seen to display either continuous or intermittent spermatogenetic activity over the course of a year.

From that point until December, the ovaries of the animals in the area produce nearly no mature eggs. In order to procreate in May and June, Bufo viridis in the Dharwar region come out of hibernation by the end of April. The majority of oocyte maturation and vitellogenesis appear to take place during the hibernation phase.

Research on the yearly cycles of activity related to spermatogenesis and oogenesis in the testes and ovaries of amphibians has been done. Determining the habitat, behavior, and geographic distribution of Bufo viridis in Rajasthan was one of the study's objectives. Examining the seasonal variations in the activity of the ovaries and testes of mature Bufo viridis in the Aravali Range foothills was another goal. In light of these details, the current study comprised eighteen months of fieldwork and aimed to explore the reproductive habits and ecology of the current species that call Rajasthan in various areas.

Key words: Temperature, Reproduction, Spermatogonial, Spermatozoa

TESTICULAR CYCLE AND SPERMATOGENIC PATTERNS IN BUFO VIRIDIS

Due to their ectothermic (pokilothermic) nature, Bufo viridis rely mostly on water for reproduction. Temperature, precipitation, day length, and relative humidity all have a major impact on their reproductive techniques, which means they are essentially seasonal breeders. Environmental factors generate noteworthy seasonal variations in testicular morphology, which is commonly investigated in the context of testicular cycles. These changes are related to spermatogenic activity.

Gonadal mitosis, meiosis, spermatid transformation into spermatozoa, spermatozoa release, and alterations in Sertoli cells, endocrine components, and interstitial cells are only a few of the events that constitute these testicular cycles. Notwithstanding external constraints, the reproductive cycle adheres to a "innate" gametogenetic rhythm.

Bufo viridis exhibit four types of spermatogenetic activity based on their geographical distribution:

1. **Continual type**: Spermatogenesis occurs year-round.
2. **Discontinuous type**: Spermatogenesis is seasonal.
3. **Continua-discontinuous type**: Spermatogenesis depends on environmental conditions, with only the spermatid stage developing during winter.
4. **Varying type**: Spermatogenesis is discontinuous in Europe but remains continuous in the Mediterranean region.

These variations in spermatogenetic activity are observed across Bufo viridis populations from different geographic locations.

MATERIALS AND METHODS:

To find Bufo viridis and determine its boundaries inside Rajasthan, a survey was conducted. Dead Bufo viridis seen on roadways were closely monitored. They worked well as a source to verify their dispersion in a certain area.

1. Study Sites

The central, northeastern, eastern, western, and southern districts of Rajasthan served as the study locations. The study examined the irrigated and unirrigated paddy fields in 18 cities, including Sawaimadhopur, Karauli, Tonk, Jodhpur, Nagore, Jaisalmer, Bikaner, Ganganagar, Udaipur, Chittoregarh, Pali, Sirohi, Jhunjhunu, Sikar, Kota, Baran, Jaipur, and Ajmer. The study was conducted on the campus of Rajasthan University and its surrounding districts.

2. Sampling

Using big torches and gathering nets, four hours of sampling (one time unit) were completed on each sampling day, from 3 to 7 a.m. and again from 7 to 10 p.m. for a total of five to six days of continuous sampling. From this stratified transect sampling, adults and juveniles were gathered and brought in for allometric measurements.

The current studies' primary goals were to investigate the reproductive biology of Bufo viridis in Rajasthan.

STUDY OF GEOGRAPHICAL DISTRIBUTION OF BUFO VIRIDIS IN RAJASTHAN

(a) Eastern Region
(b) Western Region
(c) Northern Region
(d) Southern Region
(e) North-Eastern Region
(f) South-Eastern Region
(g) Central Region

(II) ECOLOGY

(a) Habits and habitats
(b) Breeding habits

(III) SEASONAL CHANGES IN THE GONADS OF BUFO VIRIDIS IN HABITING JAIPUR REGION

(a) Pre-breeding season
(b) Breeding season
(c) Post breeding season

3. Maintenance of animals

To research the Bufo viridis in captivity, an artificial pond was placed inside the departmental forgery. There were a lot of little insects, green algae, and water lilies in the pond. Fish tanks, two to three feet deep, were used to house the bufo viridis. A significant number of insects were drawn to the two 200-watt electric bulbs hanging over the tanks at night, and these insects fell into the tanks, providing food for the adult insects.

4. Anaesthetization

Diethyl ether was used to anesthetize the adult males as needed. The ovaries of the mature females were removed after they had been narcotized. After being placed in tap water, the narcotized animals often recovered in five to ten minutes.

RESULT AND DISCUSSION:

Seasonal changes in the gonads of Bufo viridis

It is entirely possible that a widely dispersed species of Bufo viridis that lives in areas with such drastically disparate climates may exhibit notable variations in the gonadal activity cycles that occur in various places each year. During breeding season, the ovaries and testes contain developed sperm and eggs that are prepared for ovulation or ejaculation, respectively, in response to the right stimuli. Other periods could see the gonads in an inactive state or in the transitional phases of oogenesis and spermatogenesis, respectively.

The testes and ovaries of five adult male and five adult female Bufo viridis were taken every month for a full year from departmental frogery and Jhalana-park, respectively. The samples were examined histometrically and histologically. They were all weighed and dissected.

(a) Prebreeding season: March-Mid June.

(b) Breeding season: Mid-June-September

(c) Post breeding season: October-February

Seasonal changes in testes

a) January 2023 to March 2023: We refer to this time as the resting period. Only primary spermatogonia that are at rest are present in the testes in January along the tubules' basement membranes.

b) April 2023 to June 2023: Spermatogenesis's early stage is when spermiation and breeding often take place. Breeding phase is the term for this stage. Sperm are dispersed throughout the testes and some are embedded in the Sertoli cells.

c) July 2023 to September 2023: During this phase, which is the most active spermatogenetic phase, mature sperm can be discovered in a modest amount by early fall. There was unquestionably more spermatogonial activity in the testes of July. Compared to August, there were noticeably less sperms floating around in September's density.

d) October 2023 to December 2023: Spermatogenetic division completely stops in October and November. December is referred to as the resting period. Steroli cells contain a mature sperm bundle implanted in them.

Spermiation response hibernated and non-hibernated Bufo viridis

Strong influences on Bufo viridis reproductive cues come from abiotic variables such temperature, precipitation, snowmelt, humidity, photoperiod, barometric pressure, and food content. For high-elevation Bufo viridis species that live in seasonally harsh habitats, temperature is particularly important in determining their reproductive behaviours. The yearly movement of organisms between their habitats of spawning and hibernation, for instance, is often impacted by significant fluctuations in temperature. High-elevation Bufo viridis need a time of hibernation to prevent inadequate food supplies and lower the energy requirements of keeping their body temperature above freezing in order to withstand extended cold conditions.

During this dormant phase, one spends the winter below the frost line in a hibernacula. As the eastern lineage of the more widespread western Bufo viridis is one notable alpine sub-species that hibernates for extended periods of time. The sole high-elevation Bufo viridis species found in the Rocky Mountain's alpine and subalpine areas between 2300 and 2700 meters is the Bufo viridis, which hibernates for up to eight months before emerging from winter refugia in May or July to reproduce. Due to significant population reductions, the Bufo viridis is presently a target species for restoration efforts. because to the chytrid fungus, habitat degradation, and insufficient regulatory systems at several breeding locations across its range.

Sperm quality and quantity between hibernated and non-hibernated Bufo viridis

Hibernation is essential for triggering gametogenesis and suitable male reproductive behaviours in Bufo viridis species that are vulnerable and found in high elevations. The technique of hibernating has been supported by anecdotal reports and widespread practice, but there is little empirical data on how it affects reproduction in these species. For the Bufo viridis species frog and toads, the impact of hibernation on sperm quantity and quality was assessed in this study. Male Bufo viridis that were hibernating (n = 19) and those that weren't (n = 21) received 10 IUg-1 body weight (BW) human chorionic gonadotropin (hCG), and over the course of 24 hours, spermic peak was collected. The quantity of guys going through spermatogenesis was unaffected by hibernation, however the concentration of sperm generated by hibernating males was increased.

The three parameters used to quantify sperm quality were total motility, forward progressive motility, and quality of forward progression. The percentage of sperm displaying forward progressive motility and the quality of forward progression were much higher from hibernated toads, despite the fact that there was no change in total sperm motility between samples from hibernated and non-hibernated Bufo viridis. Our theory that hibernation affects the amount and quality of sperm in male Bufo viridis is supported by these findings.

Sperm concentration

At every time point, the hibernated male Bufo viridis group's sperm concentration was consistently larger ($F_{1,161} = 49.6$; $P < 0.001$) than that of the non-hibernated male group. Additionally, sperm concentration changed at various treatment time points after hCG was administered ($F_{6,161} = 3.81$; $P < 0.001$). For instance, sperm production in male hibernators increased considerably ($P < 0.001$; Tukey's HSD) between 2 and 9 hours after injection compared to the two later collection time periods of 12 and 24 hours. For hibernating Bufo viridis, peak sperm concentrations ranged from 4.3 to 5.2 × 106 mL−1, while for non-hibernating toads, they were 1.5–1.8×106mL−1. By the 24-hour collection period, sperm production was dramatically decreased ($P < 0.05$), with 0.6 ± 0.3 × 106 mL−1 and 1.7 ± 0.5 × 106 mL−1 for both non-hibernated samples.

Regarding sperm concentration, there was no significant treatment by time interaction ($F_{6,161} = 1.07$; $P = 0.38$), suggesting that although concentration was higher in hibernating animals (Fig. 1b), the direction and overall spermiation profile between the two groups did not change over time. Therefore, even if the number of animals spermiating and the production profile did not change over time, the percentage of sperm generated when male Bufo viridis hibernated was noticeably greater.

Spermiation response

For analysis, urine samples were successfully obtained from every guy at every collection time-point (2, 3, 5, 7, 9, 12 and 24 h PA). Prior to hormone therapy (T0), all urine samples that were taken were aspermic. In both the hibernating and non-hibernating groups, spermiation was induced in over 60% of the men as early as two hours after hormone delivery. The proportion of males responding to hormone therapy did not differ significantly ($t_{38} = 4.90$; $P > 0.05$) between hibernating and non-hibernating Bufo viridis. Regardless of the treatment, the spermiation response stayed between 74 and 84% of males over the collection times, with the exception of a drop to 57% in non-hibernated males at the 24-hour collection period.

Histology of Testes:

The average testicular weight peaked in June and then began to decline steadily until August, when it began to decline sharply until October, when it reached its lowest point. It went up a little during the course of the following month, then decreased between November and February before rising continuously until June. Testicular and seminiferous tubule average diameters showed a similar increase and fall trend. The several stages of spermatogenesis were not consistently abundant or scarce in the testes throughout the year, according to histological analysis.

The best months to find secondary spermatogonia nests were March and October; from January to April and June to August, there were less of them, and in September, November, and December, there were hardly any at all.

Primary spermatocytes were most prevalent in December and February, somewhat common in September and October, and scarce in March and August. The testes had a high concentration of secondary spermatocytes from March to July, and a comparatively low concentration throughout the other months. Throughout the year, spermatids were quite common; however, they were most prevalent from April to July and least common in September and October testes.

The testes in September and November had fewer sperm bundles than in March through August, when they were more common. Spermatozoa, including developing sperm trapped in Sertoli cells, were nearly absent from tests tested in October and from December to February.

According to the study, Bufo viridis has a continuous spermatogenetic cycle. Numerous studies have previously demonstrated this.

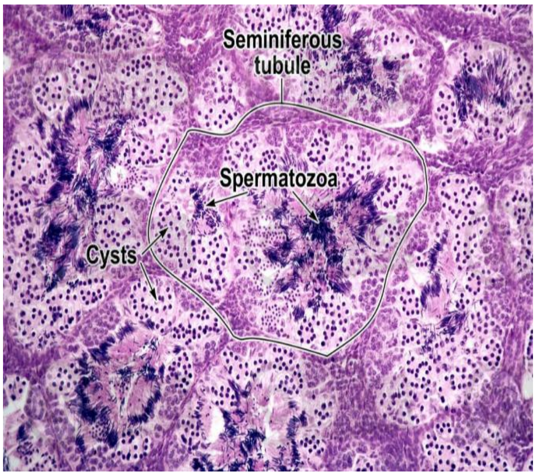

Fig-1: Slide of TS of Testes

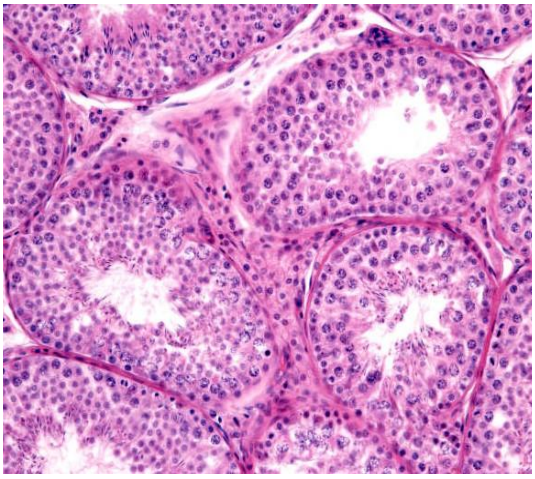

Fig-2: Slide of Seminiferous Tubules

Seasonal changes in ovaries

a) January 2023 to March 2023: There were several secondary oocyte nests in March, but fewer in January and February. Ova did not attend. In January and February, there weren't many developing oocytes.

b) April 2023 to June2023: By the end of May, vitellogenesis was finished, and the ovaries are filled with scattered eggs.

c) July 2023 to September 2023: Small primary oocytes emerge in August as a result of oogonial proliferation that occurs shortly after breeding in July. In August, the weight of the ovaries grows and the quantity of developing oocytes decreases.

d) October 2023 to December 2023: In December, ovaries did not contain primary oocytes. The quantity of fully yolky oocytes rose from October to December.

Histology of Ovary:

Every month of the year, ovaries are sectioned and histologically analyzed to reveal all phases of oocyte growth, including oogonia primordial oocytes and developing oocytes displaying varying degrees of yolk deposition. Seasons affected the relative number of oocytes at various stages of development in the ovaries. In terms of numbers, the proportion of primary oocytes climbed practically continuously from August to February, then declined continuously from March to July. Growing oocytes increased consistently in quantity from February to July, and then decreased in August and September. They multiplied once again during the course of the following month, and from October through February, their numbers were about steady.

When ovaries were collected between November and January, there were no atretic follicles. They were first seen in modest numbers in February and March, but then their numbers grew over time, peaking in September and October. In the case of July ovaries, the maximum ovarian weight per 100 gm of body weight was discovered. It dropped significantly in August and stayed roughly where it was until February.

following that, it climbed gradually until it reached its peak in July, showing that vitellogenesis causes oocytes to grow at its fastest rate following hibernation throughout the brief spring and summer before the breeding monsoon season begins.

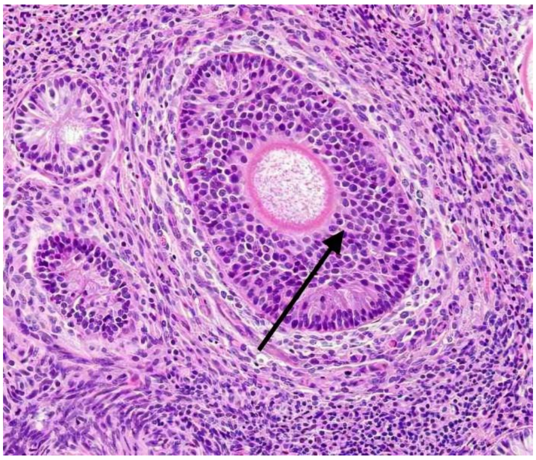

Fig-3: Slide of Ovary

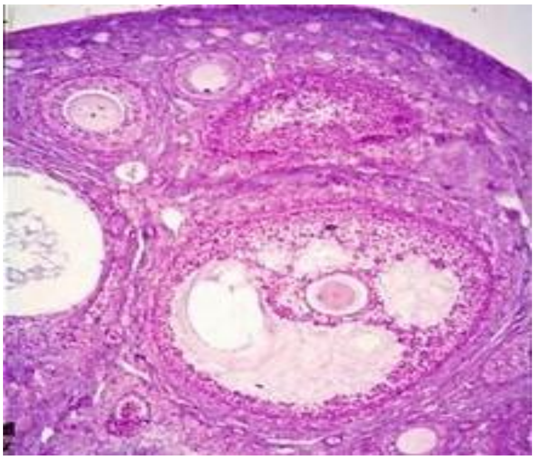

Fig-4: Follicle Slide of Ovary

CONCLUSION:

The study concludes that although spermatogenesis takes on in the testes all year round, the strength of the process fluctuates with the seasons. Even while spermatids are present in reasonable quantities during the winter (October to February), spermiogenesis the process by which spermatids turn into spermatozoa is either inhibited or does not occur at all. From March to August, when all three terminal stages secondary spermatocytes, spermatids, and spermatozoa are prevalent, the testes are at their most active. According to certain theories, the race of Bufo viridis that lives in the Rajasthan region could be categorized as "potentially continuous types" in terms of spermatogenesis.

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