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The Study of Ecology and Seasonal Variation in the Gonads Of *Bufo Melanostictus* in The Foothills of Aravali Range

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ABSTRACT
Mismanagement of agro ecosystems and denudation of habitats caused amphibians populations to numerically decrease in recent years. Change in habitat, climate etc. affected reproductive ability of the amphibians. Like other amphibians, frogs/toads are seasonal breeders and in most the reproductive activity is restricted to a certain part of the year with the gonads remaining more or less quiescent during the rest of the year. The study was aimed to find out the seasonal changes in the activity of the testes and ovaries of mature individuals of the toad/frog inhabiting in the Jaipur region.
Key words: *Amphibian, Ecosystem, Gonad, Population*

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INTRODUCTION

Amphibians are the first vertebrates to become adapted to life on land. The anurans are found in ponds, marshy ditches grassy lands, gardens etc. Frogs and toads hibernate during winter and aestivate during summer seasons. Amphibian populations have numerically decreased in recent years due to dis-management of agro ecosystems and denudation of habitats, although no published data on the community structure of amphibians in Indian ecosystems is available. On this assumption Government of India has banned the export of frog legs. Change in habitat, climate etc. affected reproductive ability of the amphibians.

Like other amphibians, frogs/toads are seasonal breeders and in most the reproductive activity is restricted to a certain part of the year with the gonads remaining more or less quiescent during the rest of the year. However in some anurans gonads have been found to be active throughout the year. Extremes to temperature is characteristic of most of the state with mean, maximum temperature during the summer months ranges from 40.5°C to 42°C, but the maximum temperature in the Arid Zone is much higher. The climatic conditions in different parts of this country vary from Alpine type in the north to tropical in south and from constantly wet and reality cooler environment of eastern region to the hot and dry desert of Rajasthan in the west. Reproduction is the fundamental process of all living things. The other so-called characteristic properties – irritability, metabolism and so on, may be absent; but reproduction is must to sustain a species [4-11].

The Aravalli range, which forms the chief topographical features in Rajasthan diagonally bisect the state into 3/5 western arid region and the 2/5 eastern semi arid zone. Climatically, Rajasthan has three seasons, summer (March to Mid June), rainy (Mid June to September) and winter (October to February). Amphibians are well represented in east of Aravallis. Many species occur in South-eastern and Southern districts – Kota, Braran, Jhalawar, Chittorgarh, Bhilwara, Rajsamand, Udaipur, Banswara, Dungarpur and Sirohi [1, 2].

With respect to spermatogenetic activity the anurans are divisible into three categories : those in which production of spermatozoa occurs throughout the year (continuous); those in which it occurs only during a certain period of the year (discontinuous) and the third category includes species in which spermatogenetic activity is at a low level during winter but it can be induced at any time by change of certain factors such as elevation of ambient temperature. The species of this category are termed as potentially continuous [22]. Three Indian anurans *Occidozyga hexadactyla, Occidozyg acyanophlyctis* and *Bufo melanostictus* are known to exhibit continuous spermatogenetic cycle [17, 18, 13]. The spermatogenetic cycle of common European frog *Rana temporaria* has been studied in detail by Van

Oordt [15]. This species is a discontinuous type in which spermatogenesis occurs following the breeding period in late summer and at its peak during June. Different races of the same species of anurans living under different climatic conditions are known to exhibit continuous or discontinuous spermatogenetic activity during a year [1]. In Dharwar (Karnataka) *Hoplobatra chustigerinus* breeds in that region in May-June and then up to December their ovaries remain almost devoid of mature oocytes. Bull frogs in Dharwar region emerge from hibernation by the end of April to breed in May-June. It would seem that the major part of the vitellogenesis and maturation of oocytes occur during the period of hibernation.

A large amount of work has been done to study the annual cycles of activity with respect to spermatogenesis and oogenesis in the testes and ovaries of amphibians, particularly the anurans, and a number of reviews have appeared on the problem [12, 13, 18, 14, 15, 19, 20, 21, 22].

One of the aims of present study was to find out geographical distribution, habit and habitat *Bufo melanostictus* in Rajasthan. Another objective was to investigate the seasonal changes in the activity of the testes and ovaries of mature individuals of the toad *Bufo melanostictus* in the foothills of Aravali Range. Keeping these facts in mind the present work involved 18 months of field work and was designed to investigate the ecology and reproductive activity of present species inhabiting Rajasthan in different regions.

MATERIALS AND METHODS

A survey was made to locate *Bufo melanostictus* and its geographical limits of Rajasthan. Toads killed on roads were observed carefully. They proved to be a good source to confirm their distribution in particular area.

Study Sites

The study sites were the central, North-Eastern, Eastern, Western & Southern districts of Rajasthan. The survey was made during December 2013 to March 2015.Total 18 cities (Sawaimadhopur, Karauli, Tonk. Jodhpur, Nagore, jaiselmer, Bikaner, Ganganagar, Udaipur, Chittoregarh, Pali, Sirohi, Jhunjhunu, Sikar, Kota, Baran, Jaipur and Ajmer) unirrigated paddy fields, irrigated paddy fields of Jhunjhunu district Rajasthan University campus were studied.

Sampling

On each sampling day 4 hours of sampling (One time unit) from 3 to 7 a.m. and again from 7 to 10 p.m. was done by using large torches and collecting nets 5 to 6 days of sampling continuously. Adult and juveniles were collected from these stratified transect sampling brought to the take allometric measurements.

The present investigations were carried out to study the reproductive biology of *Bufo melanostictus*in Rajasthan with the following main objectives:

(I) **STUDY OF GEOGRAPHICAL DISTRIBUTION OF *Bufo melanostictus* 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 melanostictus* IN HABITING JAIPUR REGION**
- (a) Prebreeding season
- (b) Breeding season
- (c) Post breeding season

Maintenance of animals

The frogs were introduced into an artificial pond in departmental forgery to study their in captivity. Pond was filled with water lilies and green algal and many small insects. Toads were kept in tanks, 2-3 feets deep. Two 200 watt electric bulbs were suspended over the tanks attracted large number of insects during nights which fell into the tanks and provided food for the adults.

Anaesthetization

Whenever needed the adult males were anaesthetized with diethyl ether. The adult females were narcotized and ovaries were dissected out. The narcotized animals normally revived in 5-10 minutes on being transferred to tap water.

Statistical analysis

Statistical calculation were based on standard method of biological statistics. Standard error was calculated using the following formula

$$\text{Standard Error (S.E.)}=\frac{\sqrt{n\sum X^2-\sum X^2}}{n^2(n-1)}$$

Histological processing

After the sampling form each study site, five adult made frogs/toads were weighed individually, calculated morphometric parameters, anaesthetized and then their testes were dissected out. Representative sections of testes for each month were photographed. Five adult female frogs/toads from among those collected each month for the histological study of the changes in the ovary.

RESULTS AND DISCUSSION

The vegetation of Rajasthan has considerable diversity on account of the variety of ecosystem found in different parts of the state. Apart from the predominant desert ecosystem, there are for example, grass lands, scrub jungles, wetlands and deciduous forests, each supporting a characteristic assemblage of plant and animal species. The great India desert engulfs the western and north western part of Rajasthan constituting the arid zone, while the southern and eastern portions merge with the more equitable climatic zones of peninsular India. This has led to wide variations both in climatic and soil conditions with the results and diversity of plant and animal life.

Geographical Distribution of *Bufo melanostictus*

The Aravali range, which forms the chief topographical features in Rajasthan diagonally bisect the state into 3/5 western arid region and the 2/5 eastern semi arid zone. Three seasons, summer (March to Mid June), rainy (Mid June to September) and winter (October to February). *Bufo melanostictus* was found to be distributed throughout the Aravali range as given below:

(a) **Eastern Region**: Tonk
(b) **Western Region:** Jodhpur
(c) **Northern Region:** Bikaner
(d) **Southern Region:**Pali and Sirohi
(e) **North-Eastern Region:**Jhunjhunu and Sikar districts.
(f) **Central Region:** Jaipur and Udaipur.

Amphibians are well represented in East of Aravali. The anurans of northeast India makeup 91% of Indian amphibians, which is just slightly more than their percentage on a worldwide basis [3, 4]. The amphibian species must have been distributed in such a manner that environmental resources are partitioned as per their requirement [2].

Ecology

The *Bufomelanostictus* were found in ponds, marshy ditches, grassy lands and garden etc. They hide in moist places, under the stones at day time, nocturnal in habit and they may be seen in paddy fields at night. Diet of anurans comprises specially orthopterous insects, dipterous nymphs, cockroaches, earthworms, spiders, ants, juveniles of its own kind, paddy grains, spiders, millipedes, centipedes, small birds, mouse, termites, mole crikckets, various hymenopterous insects, earwigs, large number of arthropod eggs, moths, dragon flies, bugs, numerous pupae and adults of mosquitoes, gastropod molluscs, lepidopterous caterpillars, seeds, grass and fibres of certain wild plants and Acacia leaves.

Breeding habits

Acoustic communications constitutes an important and conspicuous part of the breeding biology of *Bufo,* in the establishment and maintenance of territories by males, in facilitating the attraction, in courtship, and in identification of sex and reproductive states. The advertisement call of the males contains information for species recognition. The females to distinguish between heterospecifics and conspecifics use these calls. Male calls throughout the night or till amplexus are reached, without changing its call pattern. It increases the intensity of its call after their response of the female. The call contains more acoustical energy, which is indicative of good physical condition and the responding female chooses this male. The female frogs were large than the males, during amplexus, the male frog grasped the female by her axilla, amplecting time in Uperodonsystoma, was 8-10 hours, the eggs are laid in masses, blackish in colour, surrounded by white foamy jelly mass. All activities were captured daily, three hours in morning and three hours in evening in the field.

Seasonal changes in the gonads of *Bufo melanostictus*

It is quite likely that a widely distributed frog/toad species inhabiting regions with such vastly different climatic conditions may show significant differences in the annual cycles of gonadal activity in different regions. At breeding time the testes and ovaries contain mature sperms and ova ready for ejaculation or ovulation, respectively, in response to proper stimuli. At other times the gonads may show inactive condition or transitional stages of spermatogenesis and oogenesis, respectively.

Testes and ovaries of 5 adults males and 5 adult females, respectively of *Bufo melanostictus* collected each month for a continuous period of 12 months from departmental frogery and Jhalana-park were studied histologically and histometrically. Each were 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 2014 to March 2014:** This period is called resting period. In January testes contains only resting primary spermatogonia along the basement membranes of the tubules.
b) **April 2014 to June 2014:** During this early phase of spermatogenesis spermiation and breeding normally occur. This phase is called breeding phase. The testes contain scattered sperms as well as a few which remains embedded in sertoli cells.
c) **July 2014to September 2014:** This period is the most active spermatogenetic phase and mature sperm can be found in moderate number by early fall. The spermatogonial activity was definitely of greater extent in July testes. In September the density of the sperms lying free in number was markedly less in comparison to August.
d) **October 2014 to December 2014:** In October and November spermatogenetic division cease entirely. The period of December is called resting period. Mature sperm bundle embedded in steroli cells.

Histology of Testes: The average testes weight was the highest in June, decreasing thereafter first gradually until August and then steeply to reach the lowest level in October. It rose a little during the next one month and then remained less constant between November and February after which it steadily increased until June. The same pattern of rise and fall was found in case of average diameters of testes and seminiferous tubules. Histological examination showed that the various stages of spermatogenesis were not uniformly abundant or scarce in the testes in all months of the year.

Nests of secondary spermatogonia were the most abundant in March and October, fewer in January to April and June to August and were very scarce or absent in September, November and December testes. Primary spermatocytes were most abundant during December to February, quite numerous in September and October and were present in small numbers in March to August. Secondary spermatocytes were very abundant in March to July testes and were present in relatively small numbers during the remaining months.Spematids were present in fair numbers throughout the year but were the most abundant from April to July and the least in September and October testes. Sperm bundles were abundant in March to August and were present in smaller numbers in September and November testes. Testes sampled in October and during December to February were almost devoid of spermatozoa and even of differentiating sperms embedded in Sertoli cells.

The study clearly shows that *Bufo melanostictus* exhibit continuous spermatogenetic cycle. It is already evident by several studies [17, 18, 13].

Seasonal changes in ovaries

a) **January 2014 to March 2014:** Nests of secondary oocytes were abundant in March, fewer in January and February. Ova were absent. Numbers of growing oocytes were very low in January and February.
b) **April 2014 to June2014:**Vitellogenesis was completed by the end of May, ovaries contain scattered ova.
c) **July 2014 to September 2014:** Soon after breeding in July oogonial proliferation results in the appearance of small primary oocytes in August. The ovarian weight increases, growing oocytes decrease in number during August.
d) **October 2014 to December 2014:** Primary oocytes were absent in ovaries in December. Fully yolky oocytes increased in number during October to December.

Histology of Ovary: All stages of oocytes growth including oogonia primary oocyte and growing oocytes showing various degrees of yolk deposition and present in the ovaries sectioned and histologically examined in each of the 12 months during the year. The relative abundance of oocytes at different stages of growth in the ovaries varied in different seasons. Numerically the relative percentage of Primary

oocyte almost steadily increased from August to February followed by a continuous decrease from March to July growing oocytes steadily increased in numbers from February to July and then decreased during August and September. Their number increased again during the next one month and then remained more or less constant from October to February.

Table-1: Seasonal changes in the testes of *Bufo melanostictus* reared in water

Months	No. of toads	Primary spermatocytes Mean ± SE	Secondary spermatocytes Mean ± S.E.	Primary spermatogonia Mean ± S.E.	Secondary spermatogonia Mean ± S.E.	Sperm bundle Mean ± S.E.
January	5	97 ± 1.07	00	108.2 ± 1.01	21.2 ± 0.16	44.2 ± 3.84
February	5	93 ± 1.16	8.04 ± 0.11	105.0 ± 1.09	45.4 ± 4.22	47.6 ± 4.55
March	5	85.20 ± 1.83	46.1 ± 3.60	115.2 ± 4.81	35.3 ± 2.42	23.0 ± 2.28
April	5	80.00 ± 2.14	60.4 ± 3.85	116.0 ± 4.82	54.5 ± 5.01	18.6 ± 2.05
May	5	81.00 ± 4.72	58.2 ± 3.04	132.0 ± 6.01	72.2 ± 5.83	35.7 ± 2.87
June	5	110.0 ± 1.02	86.1 ± 2.71	114.0 ± 5.39	52.6 ± 6.01	25.5 ± 2.05
July	5	136.4 ± 3.21	92.2 ± 3.21	96.80 ± 3.81	26.5 ± 3.15	28.2 ± 2.06
August	5	122.2 ± 2.42	77.1 ± 7.11	91.60 ± 3.08	49.4 ± 2.69	26.4 ± 2.69
September	5	106.0 ± 3.70	54.4 ± 0.71	86.40 ± 4.37	60.4 ± 5.02	60.3 ± 3.09
October	5	108.1 ± 1.55	57.8 ± 0.91	80.10 ± 4.65	67.4 ± 5.23	50.8 ± 5.83
November	5	103.2 ± 4.20	25.0 ± 0.16	87.2 ± 4.61	59.8 ± 3.52	54.4 ± 5.54
December	5	106.0 ± 3.07	16.0 ± 0.14	91.1 ± 3.31	50.3 ± 3.23	55.2 ± 6.54

Table-2: Seasonal changes in the various types of oocytes per ovarian section in toad, *Bufo melanostictus*

Months	No. of toads	Primary oocytes Mean ± SE	Growing oocytes Mean ± S.E.	Oogonia Mean ± S.E.
January	5	74.29 ± 2.12	6.71 ± 0.34	3.92 ± 0.870
February	5	72.22 ± 3.39	6.62 ± 0.71	3.18 ± 0.053
March	5	67.12 ± 4.12	7.44 ± 0.48	1.89 ± 0.130
April	5	62.32 ± 1.50	9.38 ± 0.68	1.88 ± 0.150
May	5	52.12 ± 4.99	8.50 ± 0.79	2.48 ± 0.010
June	5	43.21 ± 1.42	8.20 ± 0.59	3.42 ± 0.050
July	5	42.44 ± 2.14	6.43 ± 0.32	3.52 ± 0.080
August	5	54.12 ± 4.19	5.49 ± 0.62	1.86 ± 0.170
September	5	62.48 ± 2.32	4.29 ± 0.37	2.37 ± 0.050
October	5	53.22 ± 5.42	5.52 ± 0.42	2.12 ± 0.001
November	5	70.70 ± 3.18	8.70 ± 0.19	2.12 ± 0.002
December	5	70.85 ± 3.11	9.87 ± 0.82	2.29 ± 0.010

Table-3: Monthly variation in the temperature, relative humidity & rainfall at Jaipur from 2014-15

Months	2014							2015						
	Temperature °C				Mean R.H. %		Total Rainfall (mm)	Temperature °C				Mean R.H. %		Total Rainfall (mm)
	High Max.	Low Min.	Mean Max.	Mean Min.	0830 IST	1730 IST		High Max.	Low Min.	Mean Max.	Mean Min.	0830 IST	1730 IST	
January	25.8	4.6	79	29	24.7	3.8	72	26.9	02.5	22.9	06.9	67	30	000.8
February	25.4	7.1	55	19	28.6	8.1	53	31.9	04.4	27.3	11.3	51	20	000.0
March	31.5	12.4	46	15	31.8	16.9	43	35.7	10.3	32.1	16.4	38	15	002.7
April	38.6	20.4	40	18	36.1	18.5	43	43.3	16.6	36.7	21.3	34	17	016.2
May	36.8	25.6	63	27	38.0	25.5	55	45.2	19.4	40.0	27.1	45	27	000.0
June	36.1	26.1	66	51	33.6	22.4	75	39.8	22.2	37.1	25.8	64	52	104.9
July	30.9	23.6	83	79	28.2	23.5	89	35.1	23.0	31.8	24.9	85	72	324.1
August	30.2	23.4	84	74	28.6	22.7	88	34.9	22.5	33.2	24.5	79	62	100.3
September	28.1	29.8	83	49	33.2	19.7	76	38.4	20.6	35.4	24.1	55	33	002.5
October	35.4	14.4	67	31	34.1	16.5	79	37.8	16.2	35.0	20.3	58	35	048.5
November	30.8	10.7	72	23	30.1	9.5	71	36.4	09.0	30.3	13.3	52	29	000.0
December	28.0	5.2	64	22	27.4	5.7	75	30.1	05.0	26.5	09.1	66	34	000.0

Atretic follicles were absent in ovaries sampled from November to January. They were present in small numbers in February and March ovaries after which their number increased gradually reaching the highest level in September-October.The maximum weight of ovary per 100 gm body weight was found in case of July ovaries. It as much lower in August remaining at approximately the same level until February. Thereafter it steadily increased to reach the highest in July indicating that the most rapid growth of oocytes due to vitellogenesis occurs after the hibernation during the short spring and summer upto the start of breeding monsoon season.

CONCLUSION

It is concluded that while spermatogenesis occurs in the testes throughout the year its intensity varies in different seasons. Spermiogenesis (transformation of spermatids into spermatozoa) is inhibited or does not occur at all during winter (October to February) although spermatids are present in fair numbers in this period. The testes are most active during March to August when all the three terminal stages (secondary spermatocytes, spermatids and spermatozoa) are abundant. It is suggested that the race of Bufomelanostictus inhabiting Jaipur region may be considered as belonging to the category of "potentially continuous types" with respect to spermatogenesis.

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