

SUMMARY

GONADAL DIFFERENTIATION AND SPERMATOGENETIC
ACTIVITY IN HIMALAYAN TOAD *Bufo himalayanas*
(Gunther) : ANURA: AMPHIBIA
– AN ULTRASTRUCTURAL STUDY

Bufo himalayanas, the material for this study is an endemic and endangered species of toad that exists in the high altitude areas of the greater Himalayan range. In my present study observations have been made on the adult and larval forms of this species, all of which were collected from different places of their normal habitat ranging between 1500 meters and 2200 meters altitude at Darjeeling town and its adjoining areas. The natural developmental processes of this toad have been monitored in laboratory. Atmospheric temperature was recorded at the time of collection, which showed a clear range between 16⁰-22⁰ C in different seasons of the year.

A normal table of development of this species, which was not done precisely before has been worked out and tabulated in a table to observe the pattern of development and the climax of metamorphosis. Statistical analysis were also done to reveal significances of their developmental pattern. It has been observed that the normal table of development has a close similarity with other closely related anuran species. Observations further confirmed that the effect of environmental temperature plays a very significant role in their developmental process. Preferably the temperature for their normal development under laboratory condition was recorded between 16⁰-22⁰ centigrade. The duration of total larval development up to the final meramorphosis is nearly two and half month. At slightly higher temperature range between 22⁰-24⁰ centigrade, this duration of metamorphosis can be

shortened. In laboratory, their pattern of growth, feeding habit, morphological features and sizes also recorded. Detailed study was made specially of the post-hatching larvae of *Bufo himalayanas*.

In the field, observations were made on the behavior and courtship pattern of the adult male and female of this species. Their eggs and larval developmental stages and characteristic features of the mature male and female were also recorded.

The study was planned and framed accordingly to draw a detailed account of larval development of this species to compare it with other related species and other closely associated classes of animal to show any significant relations with them from the evolutionary or phylogenetic point of view. For this purpose a number of related references consulted. All these were incorporated in the first chapter, named as "NORMAL TABLE OF DEVELOPMENT". The normal table was made according to the standard proposed by Gosner (1960).

In the second chapter 'GONADAL DIFFERENTIATION PATTERN AND TESTICULAR DEVELOPMENT' has been observed through light, scanning and transmission electron microscopy to reveal their structural and ultra-structural details. These observations indicated that there was a similarity in developmental pattern of gonads as well as testes with that of teleosts and reptiles which has a clear indication on their phylogenetic relationships between closely related groups of animals.

When observing the mechanism of gonadal differentiation, the evidence of bi-potential nature of differentiating gonads were also recorded. Furthermore it has been observed that developing gonads of this species has two distinct areas as peripheral cortex and inner medullary zones. The proliferation of

the cortical cells with a medullary regression marks the transition into a presumptive ovary in a female embryo, while the reverse occurs in the male.

Like many other anurans, the testes of this species differentiate and mature well before metamorphic climax (as early as four limb stage of developing larva). Thus the study further suggested, through observations, that there is a clear case of progenesis in this species.

Third chapter is concerned with the 'EVENTS OF SPERMATOGENESIS' with the aid of structural and ultra-structural observations made through light, scanning and transmission electron microscopy. A lot of unique and characteristic structural observations have been made. Here in this species, spermatogonial cells with hypo- and hyperdiploid configurations have been observed. Further observations revealed polyploidy along with polymorphic sperms in this species.

In the developing spermatogonia it has been observed that the spermatogenesis is of cystic type like other amphibians. Different stages of spermatogenesis were also recorded. Here in this species stages like primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids and mature spermatozoa have been found. Detailed light and electron microscopical observations revealed structural and ultra-structural characteristics of the cells. Here in this species a perforatorium, which consists of a number of coarse dense strands and located at the end of the nucleus of the mature spermatozoa has been recorded. Observation also confirms the presence of a bi-flagellate tail with an undulating membrane in between, except the posterior end.

The fourth chapter 'SPERM POLYMORPHISM' deals with this unique feature of polymorphism of spermatozoa in this species. The structural and ultra-structural peculiarities have been observed in the mature spermatozoa

of this species. Further study confirms that the mature sperm of this species has a characteristic biflagellate appearance. It has along slender head followed by a short mid-piece.

Transmission electron microscopic observations reveal that the electron dense materials are spread in the entire nucleus and have a different degree of compactness at various levels. Nucleus has, at its anterior end an acrosomal cap of barb like appearance with several discontinuous filaments in it. The elongated middle part has a electron lucent vacuole surrounded by heavily packed multilayer of conspicuous and circular mitochondria. The middle part also has two distinct centrioles.

The tail is uniquely bi-flagellate and each flagellum consists of an axial filament consisting of (18+2) sub-microscopic longitudinal microfibrils. Both types of electron microscopies show different forms of atypical spermatozoa like megacephalic, globular, sickle shaped and microcephalic sperm head.

The fifth and final chapter named as “ THE KINETICS OF SPERMATOGENESIS” observed the results of autoradiographic study to know the kinetics of spermatogenesis and spermiogenesis in particular. It shows that the total duration of spermiogenesis in this species is about 9.5 days and the kinetics of the total process of spermatogenesis is about 29 days which has a close similarity with related species as well as other amphibian, teleost and reptilian species which may has a strong phylogenetic significance.

The present investigation also suggests that temperature plays an important role in meiotic transition and spermatogenesis. The degeneration of pachytene spermatocytes indicate the arrest of further development during winter season of low temperature suggest that below 10⁰ C meiotic transition

retards and so spermatids and spermatozoa are not formed in this high altitude dwelling species.

Kinetics of spermatogenesis shows rapid transition and completion of spermatogenesis within the short normal breeding season (May- August). Seasonal variation observed in this species due to temperature difference of the various seasons which also has a similarity with other high altitude species of anurans.

Therefore the entire present study revealed a lot of characteristics of the normal table of development, gonadal differentiation pattern as well as testicular development. Various events of spermatogenesis, sperm polymorphism and the kinetics of the spermatogenesis of this high altitude dwelling endemic species of toad found in the Himalayan range only are also observed. Observation shows similarities with the other closely related species as well as classes of animal which suggest a definite phylogenetic significance of the present study. Recorded observations indicate the development as well as structural characteristics of this unique species of toad, which has not been done before and importance of further study specially in the field conditions.