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Determination Of Age, Longevity And Age At Sexual Maturity In Common Asian Toad (*Duttaphrynus Melanostictus*) By Skeletochronology

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ABSTRACT

Skeletochronological estimation of age, longevity and age at sexual maturity of male common Asian toad, (*Duttaphrynus melanostictus*) was reported. Toads (n=67) of different sizes were collected locally during June-November of the year 2015-2016 and brought to the laboratory. After studying their secondary sexual characters, snout-vent length and body weight of each toad was recorded. Fourth phalanx of 4th toe of hind limbs, femur and humerus bones were processed for Skeletochronology. Cross sections of bones and phalanges showed growth zones and LAGs corresponding first growth and arrested growth during summer and winter seasons respectively. Young and immature toads were without LAGs and considered to be less than one years old. Toads with 1 to 5 LAGs were sexually matured and 2 to 6 years old. The results suggest that this toad attains sexual maturity during 2nd year of their life cycle and may live for a maximum of 6 years in their natural population.

INTRODUCTION

The accurate determination of age of the individual is necessary in studies concerning growth, age at maturity, age structure in natural population, making plan for conservation and gerontology. In species capable of breeding in captivity, it is easy to know the age by keeping the birth record. But for animals, which breed favorably in nature, some alternative methods have to be followed to assess the age of the individual. Mark and recapture method was one of the methods of age determination in amphibians. But the process is tedious and time consuming. On the other hand use of bone histology based on skeletochronology technique dealing with hard tissues of vertebrates in general (Castanet, 1975, 1978) has been recognized as the most meaningful and practice able method not only to access individual's age but also the speed of growth, age at sexual maturity and the longevity of various species.

The actual age can be determined by counting annual rings in the compact bone of the cortex of amphibians and reptiles (Saint Girons, 1965). Growth lines are also found in scale and skeleton (cross sections of spines and fins) of fishes (Quasim and Bhatt 1966; Seemakula and Larkin, 1968), cross section of diaphysis of long bones (humerus and femur) of amphibians (Grifths, 1961), Smirina, 1972; Castanet, 1975; Smirina and Rocek, 1976).Bones of Lizards (Peabody, 1961; Warren, 1963; Smirina, 1974; Castanet, 1978; Patnaik and Behera 1981; Mohapatra et al., 1989), Turtles (Castanet and Cheylan 1979) and snakes (Bryurgin 1939; Peaboy 1961) have been shown to exhibit conspicuous growth makes which could be successfully employed for age determination in respective species. These marks are variously known as cortical banding or cortical lamination, resting lines or lines of arrested growth (LAG) (Smirina 1983).

The formation of annual layers reflects the seasonal changes in the growth rate of an animal. The spring - summer period of growth leads to form a wide band of bone tissue and the autumn - winter cessation of growth to a resting line. Castanet et al., (1977) suggested to call these parts of annual layer as "Mark of Skeletal Growth"(MSG) and "Line of Arrested Growth" (LAG) respectively. The annual formation of bone growth layers has been confirmed in a number of bones, that includes : Parasphenoid, Zygapophyses of Vertebrae, long tubular bones as well as in phalanges in amphibians (Smirina, 1994). Counting of annual rings in bone tissue has been taken as the routine method of age determination of amphibians (Smirina, 1994; Esteban et.al., 1996; Kumbar and Pancharatna 2004, Nayak et al., 2008). Formation of annual bone growth layers in amphibians and reptiles has also been confirmed by many earlier workers (Francillon, 1980, Ishchenko and Ledenstov, 1987; Castanet, 1975). So Castanet (1994) has rightly suggested that skeletochronology is an operational and reliable tool for age estimation in most of the living and extinct species of lizards. Cross-sections of decalcified phalanges of amphibians and reptiles stained with haematoxylin (skeletochronology) reveal growth rings in a more convincing manner, and the method is believed to be a reliable indicator of age with 90 – 95% accuracy (Patnaik, 1994).

Studies on the determination of age and longevity of amphibians through skeletochronology have been confined to mostly temperate species (Hemelaar 1983; Castanet and Smirina, 1990; Smirina, 1994; Esteban et al., 1996). Similar studies on species inhabiting warmer areas or the tropics are scanty (Esteban et al., 1996). However, some of the past works on different tropical amphibian species particularly on anurans have also been reported earlier. (Kulkarni & Pancharatna, 1996; Pancharatna et al., 2000; Kumbar and Pancharatna, 2001a, 2001b; Pancharatna and Deshpande, 2003; Kumbar and Pancharatna, 2004; Nayak et al., 2008).

It has been suggested that distinct summer and winter climates may be entirely responsible for the development of distinct growth marks (Pancharatna, 1994) due to active and slow growth rate respectively. This distinction may not be clear in all areas of tropical / subtropical regions. This has tempted us to undertake this work with common male Asian toad inhabiting in and around Paralakhemundi locality of the Gajapati district of Southern Odisha adjacent to the Eastern Ghats and accordingly to use the age structure for other studies.

MATERIALS AND METHODS

Male common Asian toads(n=67) of different sizes (snout-to-vent length- 2.5 cm. to 9.9 cm.) collected from the Paralakhemundi locality (18º 45′ north latitude, 84º 6′ east longitudes) of Odisha, India during June to November month of the year 2010-2012 were used for age determination using skeletochronology technique.

Skeletochronological studies were conducted in those toads using long bones (humerus and femur) and the longest phalanges of the hind limb as reported by Patnaik and Behera (1981) for Calotes versicolor. The animal was killed by a blow on the head, then the long bones and phalanges were dissected out and preserved overnight in 10% formalin. The bones were cleaned in running tap water for 24 hours and then decalcified in 10% EDTA (Ethylene diamine tetra acetate, Disodium salt) solution at room temperature. The period of decalcification varied from 07–32 hours depending on the size of the toad from which the bones were collected. The bones were then washed under running tap water over night. The decalcified bones were dehydrated through a graded series of alcohol (30%-100%) and processed for paraffin wax (melting point: 56–60oC) block preparation. The diaphysis portion of long bones (femur) and the fourth phalanx of the fourth toe were used for cutting of serial sections (6 – 10) in a rotary microtome. The sections obtained were stained using Delafield haematoxylin and eosin as reported earlier (Hamelaar, 1983; Patnaik and Behera, 1981) and examined under compound microscope. Photographs were obtained from appropriate sections using pentax-k 1000 camera.

RESULTS AND DISCUSSION

Male common Asian toads(n=67) of different sizes (snout-to-vent length- 2.5 cm. to 9.9 cm.) collected from the Paralakhemundi locality (18º 45′ north latitude, 84º 6′ east longitudes) of Odisha, India during June to November month of the year 2010-2012 were used for age determination using skeletochronology technique.

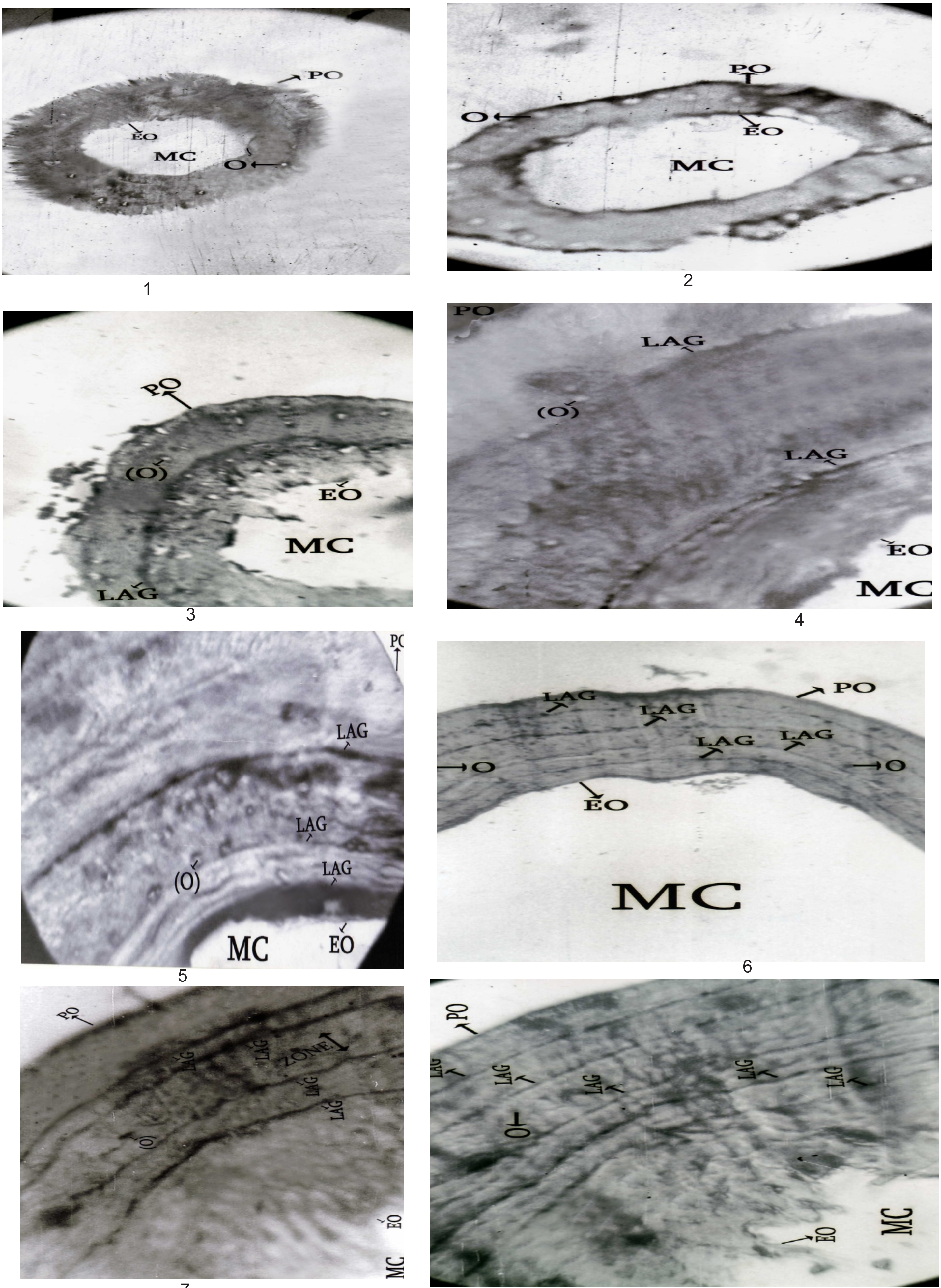

Figure 1 Cross section of femur of male D. *melanostictus* of s-v length 3.0 cm. showing no LAG(Plate No 1),of s-v length 4.0 cm. showing no LAG(Plate No 2), of s-v length 5.8 cm. showing one LAG(Plate No 3), of s-v length 7.5 cm. showing two LAGs(Plate No 4), of s-v length 8.0 cm. showing three LAGs(Plate No 5), of s-v length 9.0 cm. showing four LAGs (Plate No 6). Cross section of phalanges of male D. melanostictus of s-v length 9.0 cm. showing four LAGs(Plate No 7), of s-v length 9.9 cm. showing five LAGs(Plate No 8).Abbreviations used: MC-Marrow cavity, EO-Endosteum, PO-Periostium, O-Osteocytes, LAG-Lines of arrested growth.

Table 1: Age determination from LAGs (Lines of arrested growth),detected on the cross section of femurs and phalanges of male common Asian toad, *Duttaphrynus melanostictus*.

Sl. No.	Snout to vent length (cm)	Range of Body weight (g.)	Number LAGs	Estimated Age (Year)
1	2.5	2.5-2.9	0 (6)	< 1
2	3.0	3.0-3.5	0 (6)	< 1
3	3.8	4.0-4.9	0 (7)	< 1
4	4.0	5.8-7.0	0 (6)	<1
5	5.0	10.0-13.0	0 (5)	<1
6	5.6	14.0-16.0	0 (5)	1
7	5.8	17.0-19.0	1 (5)	1+
8*	6.0	19.0-22.0	1 (5)	2
9*	6.5	24.0-27.0	2 (4)	2+
10*	7.5	38.0-42.0	2 (3)	3
11*	8.0	44.0-47.0	3 (4)	3+
12	8.3	48.0-50.0	3 (3)	4
13*	8.5	51.0-54.0	4 (3)	4+
14*	8.8	55.0-58.0	4 (2)	5
15	9.0	60.0	4 (1)	5
16	9.3	62.0	5 (1)	5+
17	9.9	65.0	5 (1)	6

Indicates male toad captured from amplecting pair. Number in parentheses indicate animals used.

RESULTS AND DISCUSSION

As in many species of reptiles and amphibians the cross-section through mid-diaphyseal region of femur bone of male common Asian toad (*Duttaphrynus malanostictus*) showed a central marrow cavity (MC), lined by an endosteal layer (EO). The middle layer (cortex) consisted of matrix containing evenly distributed osteocytes (O) has been bounded by an outer periosteal layer (PO) (Plate-2). In the mature toads, a series of concentric darkly stained haematoxylinophilic lines separated by wider lighter zones were observed in the matrix (Plate-6). These darker lines are known as the lines of arrested growth (LAG) and the broader light colored zones as growth zones. Each growth zone along with LAG was considered as an annual ring.

The osteocytes are confined to the thicker zone of the cortex, whereas the thinner area containing LAG is devoid of them. The nuclei of osteocytes and the LAG regions are haematoxylinophilic . In young and middle-age group of specimens the size of osteocytes are relatively larger than old specimens (Plate-2, 4 and 8). The relative abundance of osteocytes in cortical region seems to be greater in the young than in the old (Plate-4 and Plate-8) toads.

Skeletochronology is considered to be the most reliable and powerful tool to estimate the age and longevity of amphibians (Castanet & Smirina 1990; Smirina, 1994; Kumbar and Pancharatna 2001a). Histology of long bones and phalanges of different anuran species revealed growth marks consisting of alternate growth zones (generally formed due to faster growth of bone) and LAGs (that reflect slower or arrested bone growth). Experimental studies have confirmed that the formation of these bone growth marks is cyclic and annual in both temperate and tropical anurans (Smirina 1994; wake and Castanet 1995; Esteban et al., 1996). Several temperate anurans like Rana temporaria (Sminira, 1972),Bufo americanus (Kalb and Zug, 1990) have been studied. All of these investigations were based on skeletochronological analysis of phalanges of captured and recaptured animals for several successive years. Few tropical anurans showing annual growth formation include Rana perezi (Esteban et al., 1996). Rana cyanophlyctis (Kumbar and Pancharatna, 2002).

The skeletochronological comparison of the initially clipped toes with toes those clipped after one year, revealed an additional LAG in the phalanges which confirmed the annual cyclicity in formation of LAG in E. cyanophlyctis and other tropical anurans (Kumbar and Pancharatna, 2004; Pancharatna and Daspande, 2003). Pancharatna and Daspande (2003) have suggested that the LAGs are laid down in the wet rainy months of the year (March – September) in tropical anurans unlike the temperate anurans in which LAGs usually laid down during winter or colder months of the year. Since these LAGs represent the number of bone growth cycles that the animal has experienced, they are used as indices of aging. So by enumerating the bone growth marks in either the phalanges or long bones, age has been estimated for a number of amphibians (Castanet and Smirina, 1990; Smirina, 1994; Esteban et al., 1996, Kumbar and Pancharatna 2001a, 2001b; Pancharatna and Despande, 2003; Kumbar and Pancharatna, 2004).

In the present study, histology of both the long bones and phalanges in male common Asian toad (Duttaphrynus melanostictus) revealed identical number of LAGs (Plate No-6 & 7) in the same individual. Similar observations have also been reported from both temperate (Bufo bufo, Mantidactylus microtympanum, Bufo americanus) and tropical (E.cyanophlyctis, Microhyla ornata, L. limnocharis) species (Kalb & Zug 1990; Kumbar and Pancharatna 2001a, 2001b; Pancharatna and Despande, 2003; Kumbar and Pancharatna 2004.

In anurans the hatching of tadpoles is accepted as age 0 and a LAG is formed each winter while periosteal growth begins a new each spring (Kalb and Zug 1990). By the end of spring frogs as well as toads usually mature and become ready for breeding activities in the coming monsoon. So the age of frogs or toads with one LAG is considered as more than one year as they have completed one year of growth. Absence of growth rings (LAGs) in immature frogs shows that the animals have not gone through the dormant stage of winter when LAGs are formed in the bone (Kalb & Zug 1990, Marnell 1997). Ages of these individuals were considered to be within one year. It is normally the number of winters an animal has survived, is counted rather than the true age of the animal. So, some researchers interpret the skeletochronological age estimation in amphibian as 1+, 2+ or 3+ years (kalb & Zug 1990; Marnell 1997) instead of 1,2 or 3 years respectively.

In the present study young male toad-lets up to s-v length 5.6 cm and body weight 15g.were found sexually immature (appearance of no secondary sexual character) and were without any LAG (Plate No 1 & 2). Study of their bone histology has revealed that the cortex of smaller and immature toad-lets is thinner than that of larger immature toads (Plate No-1& 2). So, during the process of growth with increase in body size bones also become thicker. Age of these toads were considered to be within one year (Table No-1).

Similarly smallest mature (with well developed secondary sexual character and well developed testes) male toad with s-v length 5.8cm and body weight 18g., showed one LAG in its cross section of femur (plate No - 3) indicating its age to be 1+ year or approximately two years (Table No--1). This is in good agreement with the previous reports of kumbar and Pancharatna, 2001a, 2001b, Pancharatna and Despande 2003; kumbar and Pancharatna, 2004 in which they have reported that the frogs (Microhyla ornata) showing one LAG are in the second year of growth or two years old. In this study none of the frogs were found to be mature without LAG and all the frogs having LAGs were matured. So it is concluded that sexual maturity in this species is attained during the second year of their growth i.e when they are with only one LAG in their bones. Similar findings have also been reported in other tropical anurans i.e Microhyla ornata (Kumbar and Pancharatna, 2001b) R.cyanophlyctis (Kulkarni and Pancharatna, 1996).

Mature male toads with s-v length 5.8 cm. to 9.9 cm and body weight 15g.to 65g. were with 1 to 5 LAGs in their bones (Plate No 3 to 8) . So they are considered to be 2 to 6 years old (Table No 1). In this study largest male toad of s-v length 9.9cm.and body weight 65g.was with 5 LAGs in its phalanx (Plate No 8), indicating its age to be 5+ years or 6 years. Since it was the toad with maximum number of LAGs, was also considered to be the oldest one. So the longevity of male common Asian toad (Duttaphrynus melanostictus) is estimated to be 6 years based on the present study. Presence of 5 LAGs were also observed in toads with s-v length 9.3cm. and 9.5 cm. indicating their age to be 6 years (Table No.1) . Longevity of other tropical anurans has been described as 5 years for M.ornata (Kumbar and Pancharatna 2001b) and 4 years for Fejervarya limnocharis (Pancharatna and Despande 2003).

Two distinct types of osteocytes i.e. the larger and smaller osteocytes were observed in the cortical layer of bones. In younger toads (up to S-V length 8.3 cm) many smaller osteocytes and some larger osteocytes were distributed throughout the cortex (Plate No. 1 to 5) . But in older toads (from S-V length 8.5cm to 9.9cm) there was gradual reduction in number of larger osteocytes and even in some old males they were altogether absent (Plate No.6 to 8). Reduction in size and number of large osteocytes may be due to cell degeneration in old age. This type of reduction in size and number of osteocytes has been reported earlier in some reptiles i.e Iguana (Enlow and Brown, 1969), Calotes versicolor (Patnaik and Behera 1981). So individuals with S-V length 8.5cm to 9.9cm (5 to 6 years old) are considered to be old ones and other matured but comparatively younger toads i.e. with S-V length 5.8cm to 8.3cm (2 to 4 Years old) are considered to be middle aged toads.

In conclusion, the present study reveals that:
(I) In natural population, male common Asian toad (*Duttaphrynus melanostictus*) lives for a maximum of 6 years.
(ii) Sexual maturity is attained in the second year of their life cycle .
(iii) Entire life span of this species consist of
a. Young and Immature stage(<1year)
b. Mature and middle aged (2 to 4 years) and
c. Mature but old. (5 to 6 years) stage.

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REFERENCES

1. Bryuzgin, V. L. (1939): A procedure for investigating age and growth in reptilia. Dokl. Akad. Nauk SSSR (N.S.), 23 : 403-405.
2. Castanet, J. (1975) : Quelques observations sur la presence et la structure des marques squelettiques de croissance chez les amphibiens. Bull Soc. Zool. Fr., 100 : 603-620.
3. Castanet, J. (1978) : Les marques de croissance osseuse comme indicateur de lage chez les lezards. Acta zoologica., 59 : 35-48.
4. Castanet, J. (1994) : Age estimation and longevity in reptiles. Gerontology, 40 : 174-192.
5. Castanet, J. and cheylan, M. (1979) : Les marques de croissance des os et des ecailles comme indicateur de lage chez Testudo hermanni et Testudo graeca (Reptilia, Chelonia, Testudinidae). Can. J. Zool., 57 : 1649-1665.
6. Castanet, J.; Meunier, F. S.; de Ricqles, A. (1977) : L' enregistrement de la croissance cyclique Par Le tissue asseux Chez les vertebres poikilothermes donnees comparatives et essai de synthese. Bull. Biol. Fr. Belg. T., 111 : 183-202.
7. Castanet, J. and Smirina, E.M. (1990) : Introduction to the Skeletochronological method in amphibians and reptiles. Ann. Sci. Nat. (Paris) , 11: 191-196.
8. Enlow, D. H. and Brown, S. O. (1969) : The bone of reptiles. In : Gans C. (Ed.) : Biology of the Reptilia. New York, Academic press, 1, 45-80.
9. Esteban, M.; Garcia-Paris, M. and Castanet, J. (1996) : Use of bone histology in estimating the age of frogs (Rana perezi) from a warm temperate climate area. Can. J. Zool., 74 : 1914-1921.
10. Francillon, H. (1980) : Mise en evidence experimentale du caractere annuel des lignes d'arret de croissance (LAC) chez le triton czete, Triturus cristatus (Laur). Bull. Sos. Zool. Fr., 105 : 343-347.
11. Griffiths, I. (1961) : Skeletal lamellae as an index of age in heterothermous tetrapods. Ann. Mag. Nat. Hist. (Ser-13), 4 : 449-65.
12. Hemelaar, A. (1986) : Demographic study on Bufo bufo L. (Anura, Amphibia) from different climates, by means of skeletochronology., Ph. D. Nijmegan, P. 135.
13. Hemelaar, A. S. M. (1983) : Age of Bufo bufo in amplexus over the spawning period. Oikos, 40 : 1-5.
14. Ishchenko, V. G. and Ledentsov, A. V. (1987) : Environmental influence on the dynamics of age structure of moon frog population. In : Environmental influence on population dynamics and structure in Animals (in Russian) Sverodlovsk. (Syuzyumova L. M. (Ed.) :, Acad. Sci. UNC, pp. 40-51.
15. Kalb, H. J. and Zug, G.R.(1990): Age estimates for a population of American toads Bufo americanus in Northern verginia-Brimleyana,Raleigh,N.C.;16:79-86.
16. Kulkarni, J.T. and Pancharatna, K. (1996) : Age related changes in ovarian follicular kinetic in the Indian skipper frog. J. Bio.Sc., 21 : 699-710.
17. Kumbar, S. M. and Pancharatna, K. (2001 a) : Occurrence of growth marks in the cross sections of phalanges and long bones of limbs in tropical anurans. Herpetol, Review, Clovis. , 32 : 165-167.
18. Kumbar, S. M. and Pancharatna, K.(2001b): Determination of age, longevity and reproduction of the frog Microhyla ornata by skeletochronology. J. Bio.Sc., 26 : 265-270.
19. Kumbar, S. M. and Pancharatna, K.(2002):Annual growth layers in phalanges of Indian skipper frog Rana cyanophlyctis (SCHN.)-Copeia,Washington,DC;(3):870-872.
20. Kumbar, S. M. and Pancharatna, K. (2004) : Annual formation of growth marks in a tropical amphibian. Herpetological Review, 35 : 35-37.
21. Mahapatra, N. N., Begum, K. A., Behera, H. N. and Patnaik, B. K. (1989) : Age determination in the lizard, Psammophilus dorsalis (Gray). J. Anim. Morphol. Physiol., 36 : 73-80.
22. Marnell, F.(1997):The use of phalanges for the age determination in the smooth newt, Triturus vulgaris-J. Herpetolo.Athens,Ohio;7:28-30.
23. Nayak, S. K.; Mahapatra, P.; Mohanty, R. K. and Dutta, S. K. (2008) :A skeletochronological analysis of age, growth and longevity of the Indian Green Frog Euphlyctis hexadactylus (Lesson, 1834) (Anura : Ranidae)- Herpetozoa, Wien; 20 : 99-107.
24. Pancharatna, K. (1994) : Age determination in amphibians. Indian Journal of Gerontology, 16 : 151-154.
25. Pancharatna, K. and Despande, S. A. (2003) : Skeletochronological data on age, body size and mass in the Indian Cricket frog Limnonectes limnocharis – Herpetozoa, Wien; 16 : 41-50.
26. Pancharatna, A. K.; Chandan, S. and Kumbar, S. M. (2000) : Phalangeal growth marks related to testis development in the frog Rana cyanophlyctis – Amphibia – Reptilia, Leiden., 12 : 371-379.
27. Patnaik, B. K. (1994 b): Ageing in reptiles. Gerontology, 40:200-220.
28. Patnaik, B.K. and Behera H.N. (1981): Age determination in the tropical agamid garden lizard, Calotes versicolor (Daudin), based on bone histology. Exp. Gerontol., 16 : 295-307.
29. Peabody F. E. (1961) : Annual growth Zones in vertebrates (living and fossil). J. Morphol., 108 : 11-62.
30.Qasim, S. Z. and Bhatt, V. S. (1966) : The growth of the freshwater murrel, Ophiocephalus punctatus Bloch. Hydrobiologia, 27 : 289-316.
31. Saint Girons, H. (1965) : Les critires dage chez les reptiles et leurs application a letude de la structure des populations sauvages. Terre. Vie., 112 : 342-358.
32. Semakula, S. N. and Larkin, P.A. (1968) : Age, growth, food and yield of white sturgeon (Acipenser transmonatus) of the Fraser River, Birtish Columbia. J. Fish Res. Bd. Can., 28 : 2589-2602.
33. Smirina, E. M. (1972) : Annual layers in bones of Rana temporaria. Zool. Zh., 51 : 1529-1534.
34. Smirina, E. M. (1974) : On the structure of layers in some bones of common toad and their possible use for age determination. Proc. Mordoviam State Preserve, 6 :3-103.
35. Smirina, E. M. (1983) : Age determination and retrospective body evaluation in the live common toad (Bufo bufo) (in Russian). Zool. Zh., 62 : 437-444.
36. Smirina, E. M. (1994) : Age determination and longevity in amphibians. Gerontology, 40 : 133-146.
37. Smirina, E. M. and Rocek, Z. (1976) : On the possibility of using annual bone layers of alpine newts, Triturus alpestris (Amphibia : Urodela), for their age determination. Vestn Cs. Spolec. Zool., 40 : 232-237.
38. Warren, J. W. (1963) : Growth zones in the skeleton of recent and fossil vertebrates; thesis, University of California, p. 136.
39.Wake D B and Castanet J (1995) :A skeletochronological study of growth and age in relation to adult size in Batrachoseps attenuates;J Herpetol., 29: 60-65.